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Tissue Heterogeneity of Calcium Channel Antagonist Binding Sites Labeled by [^{3}H]Nitrendipine

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SUMMARY

Calcium channel antagonist binding sites have been labeled in cerebral cortex, heart, ileum, and skeletal muscle with [^{3}H]nitrendipine. While the dissociation constants of the site from cortex, heart, and ileum are similar, $K_D \cong 0.1$–0.2 nM, the value in skeletal muscle is 2 nM. This difference in affinity is also reflected in the K_i values of dihydropyridine calcium channel antagonists, nifedipine, nimodipine, PY108068, SKF24260, and nisoldipine, and the calcium channel agonist CGP 28392, all of which show lower affinity for the skeletal muscle binding site. The diphenylalkylamine calcium channel antagonists, lidoflazine, cinnarizine, flunarizine, and prenylamine, however, show a 3- to 10-fold increase in affinity in skeletal muscle relative to the other three tissues. EDTA treatment of membranes decreases binding in cortex, heart, and ileum but increases binding in skeletal muscle. These changes are reversible upon addition of $CaCl_2$, $SrCl_2$, or $BaCl_2$. The different properties of [^{3}H]nitrendipine binding in various tissues may relate to the varying tissue sensitivity to organic calcium channel antagonists.

INTRODUCTION

Organic calcium channel antagonist drugs have important therapeutic actions in angina pectoris, cardiac arrhythmias, hypertension, and other medical conditions. These drugs fall into distinct structural classes, which differ in their pharmacological actions. Thus, while phenylalkylamines such as verapamil and benzothiazepines such as diltiazem alter cardiac rhythmicity, dihydropyridines such as nifedipine have little effect on cardiac rhythm (1). There also are considerable differences in tissue selectivity even within a given chemical class. For example, among the dihydropyridines, nimodipine appears to relax cerebral vessels more potently than peripheral arteries, while the reverse is true for nisoldipine (2, 3). The ubiquity of calcium ions as a regulatory factor requires the development of calcium antagonist drugs with tissue selectivity for the treatment of disorders of one organ system without producing untoward side effects. Thus, nimodipine has utility in relieving cerebral arterial spasm following subarachnoid hemorrhage without producing major peripheral cardiovascular influences (4). This may reflect the greater potency of nimodipine for relaxation of cerebral vessels (2, 3), although one cannot exclude other dihydropyridines having similar clinical efficacy.

Apparent receptor sites for the dihydropyridine calcium antagonists can be labeled with [^{3}H]nitrendipine (5–9), [^{3}H]nifedipine (10), or [^{3}H]nimodipine (11). While other classes of calcium antagonists do not compete directly at these sites, they do increase the dissociation rate of [^{3}H]nitrendipine in cerebral cortex (12). Phenylalkylamines such as verapamil, and diphenylalkylamines such as prenylamine allosterically decrease [^{3}H]nitrendipine binding, whereas the benzothiazepine diltiazem increases [^{3}H]nitrendipine binding (9, 12–14). In the present study we have evaluated [^{3}H]nitrendipine binding in membranes from cerebral cortex, heart, ileum, and skeletal muscle of the guinea pig. Tissue differences in the binding of the dihydropyridines and other classes of calcium antagonists and EDTA on these binding sites are described.

MATERIALS AND METHODS

Drugs were obtained from the following sources: [^{3}H]nitrendipine, New England Nuclear Corporation; nifedipine, Pfizer; SKF 24260 [1,4-dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylic acid diethyl ester], Smith Kline & French; PY108-068 [1,4-dihydro-2,6-dimethyl-4-(benzoxadiazolyle)-3,5-pyridinedicarboxylic acid diethyl ester], Sandoz; nimodipine, nisoldipine, nitrendipine, Miles; CGP28392 [4-(2-(difluormethoxy)phenyl)-1,4,5,7-tetrahydro-2-methyl-5-oxofuro (3,4-β)pyridine-3-carboxylic acid ethylester], Ciba-Geigy (Basel, Switzerland); verapamil and D-600 (gallopamil, methoxyverapamil), Knoll Pharmaceutical (Whippany, N. J.); tiapamil, Hoffmann-La Roche; flunarizine, cinnarizine, lidoflazine, Janssen Phar-

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maceutical (Beerce, Belgium); prenylamine, Hoecht-Roussel (Somerville, N. J.); diltiazem, Marion Laboratories (Kansas City, Mo.).

All drugs were prepared fresh daily in absolute ethanol at 10 mM. Appropriate dilutions were then made with 50 mM Tris-HCl (pH 7.7). All other chemicals and supplies were obtained from standard commercial sources.

Binding assays in membranes prepared from male Hartley guinea pig cerebral cortex, heart, ileum, or soleus muscle, filtration, and liquid scintillation counting were carried out as described (6). Basically, cerebral cortex, heart, ileum, and soleus muscle were removed from guinea pigs following decapitation and exsanguination. After rinsing briefly in ice-cold 0.9% NaCl, the tissues were homogenized in 50 mM Tris-HCl (pH 7.7), using a Brinkman Polytron at setting 7 for 30 sec. The homogenates were centrifuged at 20,000 rpm for 10 min in Sorvall SS-34 rotor and then resuspended in 10 volumes of 0.8–1 M KCl in 50 mM Tris-HCl (pH 7.7). The homogenates were incubated on ice for 30 min and then recentrifuged at 20,000 rpm for 20 min. KCl pretreatment allowed for higher tissue concentrations in the incubations of the muscle preparations than would otherwise be possible, presumably by dissociating actomyosin complexes (15). In order to standardize conditions, all tissues were treated similarly. This treatment caused no change in the affinity or drug specificity of the sites. The pellets were then washed three times with 50 mM Tris-HCl (pH 7.7). Following this wash, the pellets were resuspended into 100 volumes (ileum), 200 volumes (cerebral cortex and heart), or 400 volumes (skeletal muscle) of 50 mM Tris-HCl (pH 7.7). [^{3}H]Nitrendipine binding shows a broad pH optimum and does not change significantly from pH 7.0 to pH 8.0.

In view of the uncertainty of the subcellular membrane localization of the [^{3}H]nitrendipine binding sites (16–20), a total membrane population was employed. As this population may be expected to contain sarcolemma, T-tubules, sarcoplasmic reticulum, and other membrane populations, it would reflect any available [^{3}H]nitrendipine binding sites. Thus, if there were differences in [^{3}H]nitrendipine binding sites in the various membrane populations, one may discriminate this either in multiphasic saturation isotherms or competition curves.

Standard incubation conditions employed 0.2 nM [^{3}H]nitrendipine and 2 ml of the above membrane suspension at room temperature for 1 hr. In typical experiments, cortex, heart, and skeletal muscle membranes were at 0.1–0.15 mg of protein per milliliter and ileum membranes were at 0.2–0.25 mg of protein per milliliter. No change in K_D for binding sites for skeletal muscle was seen if the receptor concentration was varied from 50–150 pM during the incubation. Nonspecific binding was defined as that which occurred in the presence of 100 nM nifedipine. Preliminary experiments showed that no greater inhibition of binding occurred at 300 nM nifedipine for any of these tissues. Specific binding under these conditions was typically 2000 cpm, and nonspecific binding was 400–600 cpm for heart, cortex, and skeletal muscle. In agreement with Bolger *et al.* (8), we have found great variability in the number of binding sites in the ileum and thus in the number of specific counts per minute bound. Specific counts per minute varied from 400 to 1000 for ileum whereas nonspecific counts per minute were relatively uniform at 1200–1500.

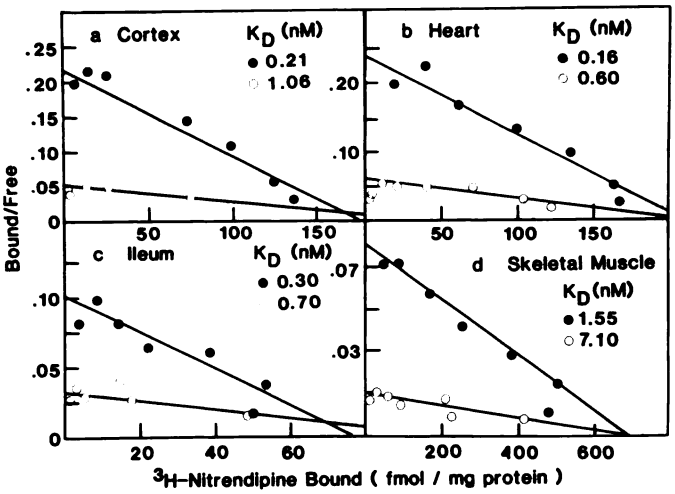

FIG. 1. *Scatchard analysis of [^{3}H]nitrendipine binding in the absence (●) or presence (○) of 1 μM verapamil*

Bound/free nitrendipine is plotted versus bound nitrendipine. Data are the means of triplicate determinations from a typical experiment that was replicated four or five times with similar results. K_D and B_{max} determinations and lines shown were obtained by linear regression. The K_D for each condition is indicated.

RESULTS

Tissue variations in dihydropyridine interactions with [^{3}H]nitrendipine binding sites. Scatchard analyses of binding with saturating concentrations of [^{3}H]nitrendipine showed a single component of binding in all of the tissues (Table 1; Fig. 1). However, the dissociation constant (K_D) varied: about 10 times higher affinity was observed in the cerebral cortex, heart, and ileum (K_D = 0.1–0.2 nM) than in skeletal muscle (K_D = 2.2 nM). Heart and cerebral cortex had similar numbers of binding sites, whereas the guinea pig ileum had only about one-half to one-third as many sites as the cerebral cortex and heart. Skeletal muscle was most enriched in binding, with B_{max} values 5 times those in the cerebral cortex or heart (Table 1).

There was a clear tissue variation in dihydropyridine affinities (Table 2). The dihydropyridines displayed similar affinities in cerebral cortex, heart, and ileum. Mon-

TABLE 1

Binding parameters of [^{3}H]nitrendipine to membranes of heart, cerebral cortex, ileum, and skeletal muscle: influences of verapamil

Dissociation constants (K_D) and maximal binding capacity (B_{max}) were determined by Scatchard analysis of saturable binding of [^{3}H] nitrendipine. Total and nonspecific binding was determined in triplicate at eight concentrations of [^{3}H]nitrendipine between 0.01 and 2.0 nM or 0.05 and 10 nM for skeletal muscle. Saturation analysis was carried out in the absence or presence of the indicated concentrations of verapamil. Values are the means ± standard error of the mean of four to seven independent determinations, carried out in triplicate.

Verapamil	Heart		Cerebral cortex		Ileum		Skeletal muscle	
	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
μM	*nM*	*fmoles/mg protein*	*nM*	*fmoles/mg protein*	*nM*	*fmoles/mg protein*	*nM*	*fmoles/mg protein*
0	0.16 ± 0.02	213 ± 32	0.20 ± 0.04	166 ± 15	0.17 ± 0.03	94 ± 20	2.28 ± 0.47	1112 ± 192
0.01	0.21 ± 0.04	147 ± 27	0.24 ± 0.03	138 ± 21	0.24 ± 0.01	52 ± 7	2.49 ± 0.40	1080 ± 210
0.1	0.51 ± 0.14[a]	148 ± 27	0.68 ± 0.14[a]	144 ± 27	0.33 ± 0.05[a]	122 ± 27	7.8 ± 2.0[a]	1020 ± 161
1	0.71 ± 0.13[a]	134 ± 15	0.57 ± 0.07[a]	139 ± 7	0.48 ± 0.04[a]	55 ± 10	8.1 ± 2.2[a]	1440 ± 45

[a] $p < 0.05$ relative to 0 verapamil.

TABLE 2

Drug specificity of [³H]nitrendipine binding in various tissues

Competition experiments using six concentrations of each drug were performed. Total and nonspecific binding in each case was defined as the binding of 0.2 nM [³H]nitrendipine in the absence or presence of 100 nM nifedipine. K_i values were determined from IC_{50} values using the relationship $K_i = IC_{50}/(1 + L/K_D)$. Values presented are mean ± standard error of the mean for K_i values or pseudo-Hill coefficients (n_H) of four independent experiments performed in triplicate.

Tissue and drug	K_i	n_H
	nM	
Heart ($n = 4$)		
Nifedipine	0.49 ± 0.09	1.00 ± 0.02
Nitrendipine	0.12 ± 0.02	1.02 ± 0.01
Nimodipine	0.28 ± 0.12	0.88 ± 0.03
Nisoldipine	0.17 ± 0.06	0.83 ± 0.03
SKF 24260	0.069 ± 0.027	0.86 ± 0.03
PY 108-068	0.15 ± 0.04	0.81 ± 0.02
CGP 28392	460 ± 100	1.14 ± 0.12
Cerebral cortex ($n = 4$)		
Nifedipine	0.67 ± 0.13	0.78 ± 0.05
Nitrendipine	0.17 ± 0.03	0.99 ± 0.04
Nimodipine	0.28 ± 0.08	0.89 ± 0.05
Nisoldipine	0.13 ± 0.02	1.06 ± 0.07
SKF 24260	0.08 ± 0.02	0.97 ± 0.08
PY 108-068	0.13 ± 0.02	1.02 ± 0.06
CGP 28392	630 ± 210	1.06 ± 0.07
Ileum ($n = 4$)		
Nifedipine	0.27 ± 0.10	0.93 ± 0.10
Nitrendipine	0.12 ± 0.05	1.13 ± 0.03
Nimodipine	0.11 ± 0.02	1.15 ± 0.17
Nisoldipine	0.07 ± 0.01	0.78 ± 0.01
SKF 24260	0.04 ± 0.01	0.91 ± 0.05
PY 108-068	0.10 ± 0.03	0.87 ± 0.09
CGP 28392	620 ± 190	1.10 ± 0.18
Skeletal muscle ($n = 4$)		
Nifedipine	2.29 ± 0.65	0.76 ± 0.09
Nitrendipine	0.64 ± 0.25	0.85 ± 0.03
Nimodipine	0.92 ± 0.21	0.99 ± 0.02
Nisoldipine	0.22 ± 0.06	1.22 ± 0.05
SKF 24260	0.17 ± 0.05	0.96 ± 0.04
PY 108-068	0.65 ± 0.13	0.99 ± 0.10
CGP 28392	1800 ± 400	1.07 ± 0.04

ophasic competition curves for all dihydropyridine drugs were obtained giving Hill coefficients of about 1.0. However, in guinea pig skeletal muscle most of the drugs were considerably less potent than in the other three tissues. This finding fits with the higher K_D value for [³H]nitrendipine binding in skeletal muscle than in cerebral cortex, heart, or ileum. In all tissues, however, the dihydropyridine binding site showed a similar rank order of potency, SKF 24260 > PY108-068 > nisoldipine > nitrendipine > nifedipine > CGP28392.

Tissue variations in regulation of [³H]nitrendipine binding by verapamil. The structurally unrelated calcium channel antagonist, verapamil, regulates [³H]nitrendipine binding allosterically, by decreasing the affinity of [³H]nitrendipine binding several-fold (9, 12). In competition curves this is seen as a plateau of inhibition of [³H]nitrendipine binding by verapamil. This plateau of inhibition of [³H]nitrendipine binding by verapamil varies with the concentration of [³H]nitrendipine. At higher [³H]nitrendipine concentrations, verapamil produces lesser inhibition, because the higher concentrations of [³H]nitrendipine can still bind despite the lowered affinity for the ³H-ligand. In the present studies a similar plateau of inhibition of [³H]nitrendipine binding was readily apparent in ileum, cerebral cortex, and heart (Fig. 2a). On the other hand, in skeletal muscle, verapamil inhibition appeared to be consistent with a competitive effect, with a pseudo-Hill coefficient of about 1.0 (Fig. 2a).

We wondered whether verapamil differed fundamentally in its interactions with [³H]nitrendipine in skeletal muscle as compared with ileum, cerebral cortex, or heart, as these interactions appeared to be competitive in skeletal muscle but allosteric in the other three tissues. However, verapamil in skeletal muscle could affect [³H]nitrendipine binding allosterically as in the other three tissues, but the higher K_D for [³H]nitrendipine in skeletal

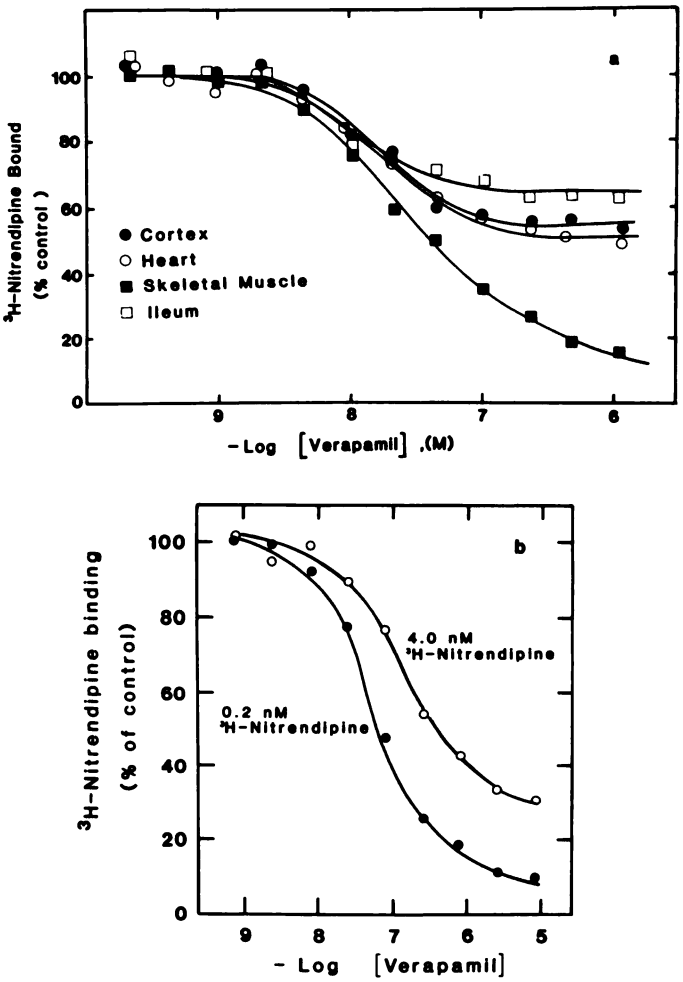

FIG. 2. *Inhibition of [³H]nitrendipine binding by verapamil in four tissues*

Total and nonspecific binding was determined as described under Materials and Methods in the absence or presence of various concentrations of verapamil in (a) four tissues (cortex ●; heart ○; skeletal muscle ■; ileum □) or (b) skeletal muscle alone. [³H]Nitrendipine concentration was (a) 0.2 nM or (b) 0.2 nM (●) or 4 nM (○). Nonspecific binding was determined in each instance with 100 nM nifedipine. Data shown are from a typical experiment that was replicated twice.

muscle would alter the competition curve by verapamil. Thus, if the [^{3}H]nitrendipine concentration is held constant at approximately the K_D for [^{3}H]nitrendipine in ileum, cerebral cortex, and heart (0.2 nM), there will be 50% receptor occupancy in these three tissues. On the other hand, 0.2 nM [^{3}H]nitrendipine is one-tenth the K_D for the ligand in skeletal muscle, so one would anticipate only about 10% receptor occupancy in skeletal muscle. One can calculate the maximal verapamil-elicited reduction in [^{3}H]nitrendipine binding if the change in K_D produced by verapamil in the different tissues is known. Accordingly, we conducted saturation analysis of [^{3}H]nitrendipine binding in the four tissues in the presence of 1 μM verapamil (Fig. 1). This concentration of verapamil increased the K_D for [^{3}H]nitrendipine 3- to 4-fold in all four tissues. Lesser reductions in [^{3}H]nitrendipine affinity occurred in the four tissues at 0.1 and 0.01 μM verapamil, except for cerebral cortex (Table 1).

Receptor occupancy of the ^{3}H-ligand can be calculated from the formula occupancy = $L/(L + K_D)$ where L is the concentration of [^{3}H]nitrendipine and K_D is the dissociation constant of the dihydropyridine binding site in the absence or presence of verapamil. In ileum, cerebral cortex, and heart preparations incubated in the presence of 0.2 nM [^{3}H]nitrendipine, one can calculate that 1 μM verapamil should reduce receptor occupancy from 50% to 28%, thus lowering [^{3}H]nitrendipine binding 44%, which is what we observed in these tissues. For skeletal muscle incubated with 0.2 nM [^{3}H]nitrendipine, the receptor occupancy in the absence of verapamil of 7.5% should be lowered by 1 μM verapamil to 2%, which goes along with the 70–80% reduction in [^{3}H]nitrendipine binding observed for skeletal muscle.

If the model for the effects of verapamil on the behavior of binding of [^{3}H]nitrendipine to skeletal muscle described above is valid, then verapamil should produce a lesser maximal inhibition of [^{3}H]nitrendipine binding with higher ^{3}H-ligand concentrations. As predicted, with higher [^{3}H]nitrendipine concentrations, the maximal inhibition by verapamil diminished (Fig. 2b). A number of calcium antagonist drugs influence [^{3}H]nitrendipine binding in a fashion similar to verapamil. Both phenylalkylamines such as D-600 and tiapamil, and diphenylalkylamines such as prenylamine, cinnarizine, lidoflazine, and flunarizine appear to inhibit [^{3}H]nitrendipine binding by increasing the rate of dissociation induced by nifedipine. This is consistent with a decreased affinity (12). As observed for verapamil in skeletal muscle, at low [^{3}H]nitrendipine concentrations, one can obtain complete displacement of the ^{3}H-ligand from cerebral cortex membranes by some of these agents, but the mechanism of inhibition is nonetheless allosteric, as it is with verapamil (12).

Like the dihydropyridines, the phenylalkylamines and diphenylalkylamines behaved similarly in cerebral cortex, heart, and ileum but differed somewhat in skeletal muscle (Table 3). D-600 was the most potent, eliciting 50% of its maximal inhibition at 6 nM. D-600 was about 4–5 times more potent than verapamil, which in turn was 10–40 times more potent than the other drugs evaluated. Some of the drugs, such as verapamil and diltiazem, had about the same potency in skeletal muscle as in the other three tissues. However, flunarizine, cinnarizine, prenylamine and lidoflazine were substantially more potent in skeletal muscle than in the other tissues.

Differential influences of cations on [^{3}H]nitrendipine binding sites in various tissues. We have previously shown that [^{3}H]nitrendipine binding in cerebral cortex membranes is regulated in a highly selective fashion by divalent cations (6). EDTA-treated cerebral cortex mem-

TABLE 3

Effects of non-dihydropyridine calcium channel antagonists on [^{3}H]nitrendipine binding

Competition experiments were performed using at least six concentrations of drug under standard incubation conditions, as described under Materials and Methods. EC_{50} was defined as that concentration which gives 50% of maximal effect. Values presented are mean ± standard error of the mean of the indicated number of experiments, which were performed in triplicate

Drug	EC_{50}			
	Heart	Cerebral cortex	Ileum	Skeletal muscle
			μM	
Verapamil	0.028 ± 0.004 (n = 4)	0.028 ± 0.003 (n = 4)	0.021 ± 0.006 (n = 4)	0.048 ± 0.011 (n = 4)
D-600	0.0012 ± 0.0006 (n = 4)	0.006 ± 0.002 (n = 5)	0.004 ± 0.004 (n = 4)	—
Tiapamil	1.2 ± 0.2 (n = 5)	1.0 ± 0.1 (n = 5)	1.06 ± 0.23 (n = 4)	0.24 ± 0.02[a] (n = 4)
Flunarizine	0.36 ± 0.08 (n = 4)	0.55 ± 0.08 (n = 6)	0.26 ± 0.09 (n = 4)	0.06 ± 0.01[a] (n = 4)
Cinnarizine	1.14 ± 0.26 (n = 5)	1.28 ± 0.26 (n = 5)	1.05 ± 0.27 (n = 4)	0.33 ± 0.08[a] (n = 4)
Lidoflazine	1.12 ± 0.32 (n = 5)	0.66 ± 0.09 (n = 5)	0.50 ± 0.19 (n = 4)	0.028 ± 0.006[a] (n = 4)
Prenylamine	0.28 ± 0.05 (n = 4)	0.25 ± 0.06 (n = 4)	0.22 ± 0.02 (n = 5)	0.017 ± 0.002[a] (n = 5)
Diltiazem[b]	0.55 ± 0.3 (n = 4)	0.47 ± 0.19 (n = 4)	—	0.32 ± 0.08 (n = 4)

[a] $p < 0.01$ relative to heart.

[b] Diltiazem stimulates binding (11–13). Thus, the EC_{50} for diltiazem is that concentration which gives 50% of maximal stimulation.

branes display little [^{3}H]nitrendipine binding. In such preparations, calcium markedly stimulates binding, and the relative ability of various cations to stimulate binding reflects their physiological actions as calcium mimicking ions (6). In the present study we compared the sensitivity to EDTA of [^{3}H]nitrendipine binding in various tissues and also monitored influences of various cations.

In cerebral cortex, heart, and ileum, increasing concentrations of EDTA progressively reduced [^{3}H]nitrendipine binding (Fig. 3). The maximal reduction at 0.1–0.5 mM EDTA of about 60% was similar in the cerebral cortex and ileum, whereas a lesser reduction of 25% occurred in heart. With higher EDTA concentrations, [^{3}H]nitrendipine binding returned toward control levels. Skeletal muscle behaved quite differently. No depression of [^{3}H]nitrendipine binding was apparent at any EDTA concentration. Instead, at 0.1–1.0 mM, EDTA stimulated [^{3}H]nitrendipine binding 40–60% (Fig. 3). Incubation with EGTA[1] gave results similar to EDTA in skeletal muscle (data not shown).

To determine whether the effects of EDTA are related to chelation of divalent cations, we added various concentrations of calcium chloride or the calcium "agonists" strontium chloride and barium chloride in the presence of 0.25 mM EDTA (Fig. 4). In a concentration-dependent fashion, calcium chloride, strontium chloride, and barium chloride reversed the EDTA-induced reduction in [^{3}H]nitrendipine binding in cerebral cortex and heart. Interestingly, they also reversed the EDTA stimulation of skeletal muscle binding.

We also compared the effects of inorganic calcium antagonists on [^{3}H]nitrendipine binding in the various tissues. Previously we showed that the calcium antagonist ions lanthanum, cobalt, copper, and manganese inhibited [^{3}H]nitrendipine binding in cerebral cortex membranes (6). In the present study we found inhibition of binding by all of these ions in the four tissues examined (data not shown).

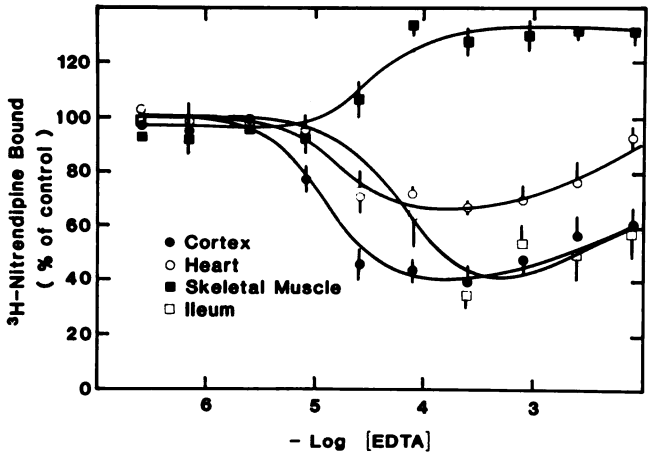

FIG. 3. *Effects of EDTA on [^{3}H]nitrendipine binding in four tissues*
Total and nonspecific binding was determined as described under Materials and Methods in the absence or presence of various concentrations of Na_4EDTA. Binding is expressed as a percentage of that occurring in the absence of added Na_4EDTA in cerebral cortex (●), heart (○), skeletal muscle (■), and ileum (□). Error bars indicate the standard error of the mean of three experiments, which were averaged. Where no error bar is shown, the standard error of the mean was less than the size of the data symbols.

[1] The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′, N′*-tetraacetic acid.

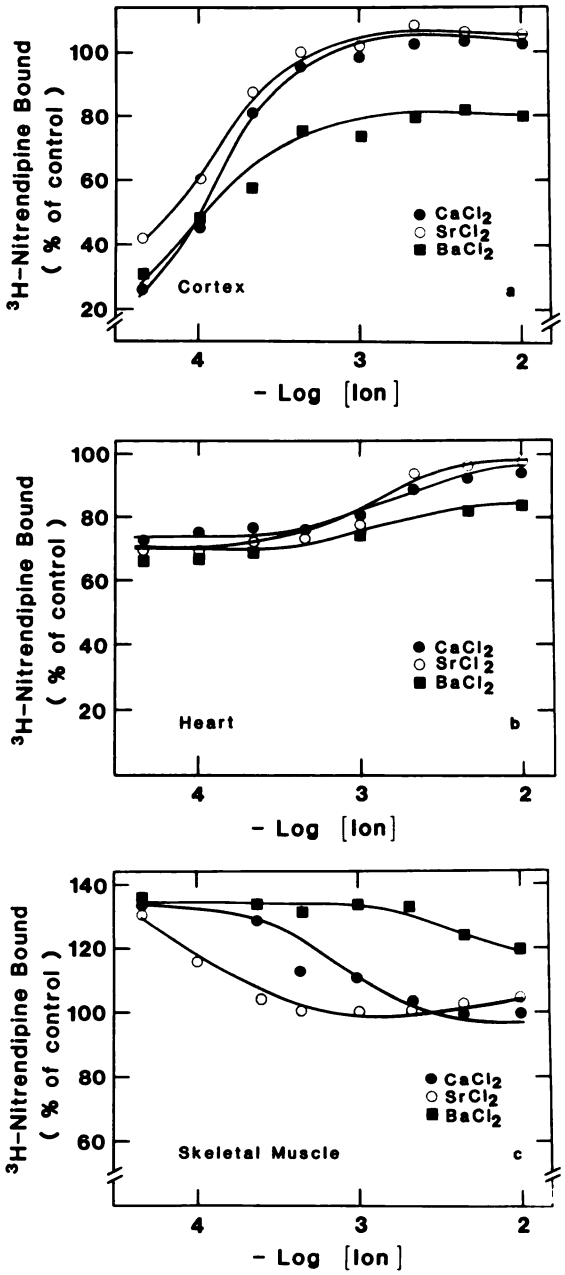

FIG. 4. *Cation reversal of EDTA effects on [^{3}H]nitrendipine binding in cerebral cortex, heart, and skeletal muscle*
Total and nonspecific binding was determined under standard assay conditions as described under Materials and Methods in the presence of 0.25mM Na_4EDTA and various concentrations of $CaCl_2$ (●). $SrCl_2$ (○), or $BaCl_2$ (■). Binding is expressed as a percentage of that occurring in the absence of any added agents for (a) cerebral cortex, (b) heart, or (c) skeletal muscle. Data depict a typical experiment which was replicated three times. The standard deviation within an experiment was less than the size of the data symbol. Experiments agreed to within 15–20%.

DISCUSSION

The major finding of the present study is the existence of tissue variations in properties of [^{3}H]nitrendipine

binding. Skeletal muscle differs in several ways from heart, ileum, and cerebral cortex. Skeletal muscle has a lesser affinity for all of the dihydropyridines evaluated, but substantially greater sensitivity of [^{3}H]nitrendipine binding to the diphenylalkylamine calcium antagonists flunarizine, cinnarizine, lidoflazine, and prenylamine as reported for heavy sarcoplasmic reticulum from skeletal muscle (16).

Skeletal muscle also has by far the highest density of [^{3}H]nitrendipine binding sites, more than 5 times the levels of the next most enriched tissue. We have conducted extensive screening of numerous body organs and blood vessels and failed to find any tissue with [^{3}H] nitrendipine binding levels more than 10% of those skeletal muscle (6).[2] The function of these binding sites in skeletal muscle is unknown. [^{3}H]Nitrendipine binding sites are linked to slow calcium channels in smooth muscle, but the relationship in other tissues is less clear (5–11). There is neurophysiological evidence that skeletal muscle possesses such channels and that these channels are weakly sensitive to organic calcium channel antagonists (21–27). However, the contractile functions of skeletal muscle are not dependent on these channels, but instead are modulated by intracellular stores of calcium. Presumably for this reason, skeletal muscle is not markedly affected in its function by organic calcium antagonist drugs (26), but in several instances, nifedipine treatment has been discontinued because of severe muscle cramps in the legs and hands (28–30). It should be pointed out that verapamil and diltiazem have not been reported to give these side effects, and a number of explanations may account for this, including the possibility that slow calcium channels of skeletal muscle may play a role under specialized conditions which have not been evaluated in connection with organic calcium antagonist drugs. In any event, the high density of [^{3}H] nitrendipine sites in skeletal muscle suggests a possible physiological role for this agent in this tissue. The high levels of skeletal muscle [^{3}H]nitrendipine binding sites are not restricted to guinea pig, as we have also found substantial binding in rat skeletal muscle (6).

The diphenylpiperazines cinnarizine, flunarizine, and lidoflazine as well as prenylamine are much more potent in inhibiting [^{3}H]nitrendipine binding in skeletal muscle than in the other three tissues. There are two possible explanations for this increased potency. These drugs do not interact directly with nitrendipine binding sites but rather act via a distinct site (12). This site in skeletal muscle may have higher affinity for the diphenylpiperazines than the similar site in other tissues. Thus, the actual differences may be in this site for verapamil-like drugs. Second, the coupling between the two sites may be different in skeletal muscle. That is, when cinnarizine, for example, binds in skeletal muscle, there is a tight coupling to the nitrendipine binding site. The efficacy in heart, cortex, and ileum would be lower.

Relative effects of cations also differentiate the various tissues. The paradoxical stimulation of [^{3}H]nitrendipine binding in skeletal muscle by EDTA is related to chelation of cations, since it is reversed by calcium, strontium, and barium. As we have not measured the endogenous levels of Ca^{2+} in our tissue preparations, it is difficult to assign quantitatively the relative efficacies of calcium, strontium, or barium. Endogenous levels of Ca^{2+} or other divalent cations at 1–10 μM would significantly alter the dose response we obtained by adding divalent cations. It is doubtful, however, that contamination of the preparation by endogenous cations would lead to the reversal of the effects of EDTA seen in skeletal muscle.

The results are in contrast to those of Fairhurst *et al.* (16), who reported no effect of EGTA at 1 mM using heavy sarcoplasmic reticulum membranes prepared from rabbit skeletal muscle. EDTA stimulates binding 20% at 0.1–1 mM (Fig. 3). We have found no difference between EDTA and EGTA at 1 mM (data not shown). Since the membrane fraction used here is heterogeneous, there may be a population of binding sites in which binding is stimulated by chelation. Heavy sarcoplasmic reticulum membranes may contain a population of sites relatively insensitive to chelation of divalent cations by EGTA or may change their characteristics during the purification (16). There is little evidence for multiple sites for [^{3}H] nitrendipine binding in these tissues. Data from saturation experiments plotted by the method of Scatchard (31) are consistently linear. Moreover, the Hill slopes of competition curves of dihydropyridines are unity, consistent with a single population of sites. The only exception is nifedipine displacement of [^{3}H]nitrendipine binding to skeletal muscle membranes, where the Hill slope is 0.65, suggestive of more than one site. However, if this is the case, nifedipine is the only dihydropyridine capable of this discrimination. This effect of EDTA represents another instance in which skeletal muscle behaves differently from the other tissues. The cerebral cortex differs from heart, ileum, and skeletal muscle in its greater sensitivity to EDTA (Fig. 3) (16).

These differences may not account for the selectivity of the dihydropyridines for various blood vessels (2, 3). We did not find any differences in the relative potencies of the various dihydropyridines in the tissues we evaluated, except in the skeletal muscle, where they are consistently less potent. In preliminary studies we detected only very low levels of [^{3}H]nitrendipine binding in pig basilar artery, canine descending abdominal aorta, and bovine meningeal microvessels and large arteries, and, thus, could not detect subtle differences in binding.[3] With total membranes from pig coronary arteries and bovine aorta, others (14, 32) have been able to detect low levels of [^{3}H]nitrendipine binding. The K_D of the nitrendipine binding sites in these arterial preparations for [^{3}H]nitrendipine is about 2 nM, similar to skeletal muscle. Greater tissue availability and higher binding levels may make skeletal muscle a good model system for nitrendipine binding sites with characteristics similar to arterial smooth muscle. Whether blood vessels from different parts of the body vary in their recognition sites for dihydropyridines or in the coupling of recognition sites to the calcium flux apparatus is unclear.

[2] R. J. Gould, K. M. M. Murphy, and S. H. Snyder, unpublished observations.

[3] K. M. M. Murphy, R. J. Gould, M. Moskowitz, A. Sastre, S. J. Peroutka, and S. H. Snyder, unpublished observations.

Behavioral effects have been described for nimodipine, which may be related to receptor sites in brain tissue other than on blood vessels (33). In autoradiographic studies, we have shown that [3H]nitrendipine binding is associated primarily with synaptic layers with very little, if any, binding detectable on blood vessels (34). Thus, drugs with calcium channel antagonist properties may be useful in a variety of neurological conditions such as affective disorders or schizophrenia (35, 36). The tissue variations in sensitivity of [3H]nitrendipine sites to calcium antagonist drugs of several classes suggest the possibility of developing agents with selectivity and attendant therapeutic utility for numerous medical conditions. Nitrendipine site heterogeneity may also reflect basic differences among voltage-dependent calcium channels from various organs or vascular beds.

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