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UPTAKE OF CALCIUM ANTAGONISTIC DRUGS INTO MUSCLES AS RELATED TO THEIR LIPID SOLUBILITIES

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Abstract—Calcium antagonists, e.g. bepridil and verapamil, block the Ca^{2+}-dependent slow action potentials in frog skeletal muscle [L. M. Kerr and N. Sperelakis, *J. Pharmac. exp. Ther.* **222**, 80 (1982)]. To determine whether the calcium antagonistic drugs may enter the fibers and exert an internal action as well, uptake of tritiated bepridil, verapamil, nitredipine, nifedipine, and diltiazem into rat extensor digitorum longus (EDL) muscles was examined. It was found that the uptake values of verapamil, nitrendipine, and bepridil were much higher than those of nifedipine and diltiazem. The order of uptake was: bepridil > nitrendipine > verapamil ≫ nifedipine > diltiazem. The small uptake values of nifedipine and diltiazem may represent primarily binding to the surface membrane. In frog skeletal muscle (sartorius) also, the uptake of bepridil was greater than that of verapamil, and disruption of the T-tubules by the glycerol method did not change them. The same order of drug uptake values was found for monolayer cultures of vascular smooth muscle cells (rat aorta). The order of uptake in isolated sarcoplasmic reticulum (SR) from rat skeletal muscles was: verapamil > nitrendipine > bepridil > nifedipine > diltiazem. The lipid solubility values of the calcium antagonists were measured by their partition coefficients in oil/Ringer, octanol/Ringer, and chloroform/Ringer systems. The order of lipid solubility was: bepridil > verapamil > nitrendipine > nifedipine ≫ diltiazem. Thus, the calcium antagonists with the highest lipid solubilities were taken up more by the muscle cells and SR. It is concluded that verapamil, bepridil, and nitrendipine enter and accumulate inside the muscle cells, whereas nifedipine and diltiazem do not permeate readily.

The inward slow Ca^{2+} currents found in cardiac muscle [1] and smooth muscle [2] are blocked by calcium antagonistic drugs, such as verapamil and bepridil, in a frequency- (use-) dependent manner. Bepridil and verapamil act in a similar manner to depress and abolish the Ca^{2+}-dependent slow action potentials in skeletal muscle [3]. Vogel *et al.* [4] demonstrated that bepridil depresses cardiac muscle contraction more than it depresses the slow inward Ca^{2+} current and suggested that bepridil enters the myocardial cells and acts to depress Ca^{2+} release from the SR. Bepridil depresses contraction of rabbit aortic rings stimulated under conditions such that the major source of Ca^{2+} for contraction is from intracellular release, and it was concluded that bepridil has a second effect on internal sites in the muscles, in addition to blockade of the slow channels [5]. Pang and Sperelakis [6] demonstrated that bepridil, verapamil, and nitrendipine enter and accumulate inside cardiac muscle and smooth muscle cells; in contrast, nifedipine and diltiazem either do not enter or permeate only very slowly.

The present experiments were undertaken to determine whether the calcium antagonistic drugs cross the surface membrane of skeletal muscle fibers and the relationship between their permeabilities and their lipid solubilities. It was found that the amount of a drug taken up by the skeletal muscle cells is directly porportional to its lipid solubility. Bepridil, verapamil, and nitrendipine readily entered and accumulated inside the cells, whereas nifedipine and diltiazem did not.

METHODS

Uptake of calcium antagonists

Rat skeletal muscle. The extensor digitorum longus (EDL) muscles from rats were incubated at 37°. The amount of radioactive calcium antagonist taken up by the skeletal muscle was determined as previously described [6, 7]. The composition of the Ringer's solution used was: 145 mM NaCl, 4.0 mM KCl, 1.0 mM $MgCl_2$, 2.0 mM $CaCl_2$, 20 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) (pH 7.4), and 10 mM glucose.

The tissue/medium (T/M) ratio was expressed as the concentration of drug taken up by the muscle at steady state with respect to the concentration of the drug in the medium (pmoles per g wet wt/pmoles per ml of medium). The protein content of the rat EDL muscle was 1.44 ± 1.4 mg/g wet wt. The $[Drug]_i/[Drug]_o$ ratio was calculated from the T/M ratio assuming that: (a) the water content of the muscle was 80% of the total wet weight, (b) the interstitial fluid was 15% of the muscle, and (c) the loss of

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radioactive label from the slow (presumably the intracellular) compartment during the 10-min wash period was 6% [7].

Monolayer cultures of vascular smooth muscle cells. Cultured vascular smooth muscle cells from rat aorta were prepared as described previously [8]. Uptake of calcium antagonistic drugs into the monolayer cultures of vascular smooth muscle cells was measured as previously described [9]. Before each assay, the culture medium was removed and the monolayers were washed with Ringer's solution. After equilibration for 5 min, the uptake experiments were started by the addition of Ringer's solution containing 10^{-7} or 10^{-6} M tritiated drug (1.0 μCi/ml). Incubation was carried out for 20 min at 37°. Uptake of drug was terminated by removing the radioactive medium and washing three times at room temperature with Ringer's solution containing 10^{-5} M cold (unlabeled) drug. The washed monolayers were solubilized with 0.1 N NaOH at 60° overnight and counted in a liquid scintillation counter. The protein content was determined by the method of Lowry *et al.* [10]. For each experiment, a zero time assay was carried out by adding the radioactive assay medium to the monolayer cultures and immediately washing the cells with the Ringer's solution containing 10^{-5} M cold drug. The "zero time" value was subtracted from the uptake values.

Frog sartorius muscle. Sartorius muscles were dissected from frogs (*Rana pipiens*) and stored at 4° in frog-Ringer's solution [133 mM NaCl, 1.88 mM KCl, 1.08 mM $CaCl_2$, 20 mM HEPES (pH 7.4), and 10 mM glucose] until used (within 14 hr). For disruption of T-tubules, the muscles were equilibrated for 60 min in 300 mM glycerol added to frog-Ringer's solution. The glycerol-containing solution was then suddenly replaced by glycerol-free Ringer's solution in order to produce sufficient osmotic shock to disrupt the T-tubules. Uptake of drugs into normal and glycerol-treated muscles was measured at room temperature. The muscles were subsequently treated in the same manner as previously described [6, 7].

Binding of calcium antagonists to sarcoplasmic reticulum of rat skeletal muscle

Sarcoplasmic reticulum (SR) from the hindlegs of rats was prepared by the method of Pang and Briggs [11]. Binding of calcium antagonistic drugs to the SR (0.1 mg/ml) was performed in the presence of 100 mM KCl, 20 mM imidazole (pH 7.0), and 10^{-7} M tritiated drugs (1 μCi/ml). Incubation was carried out at room temperature for 10 min. The SR was then collected on a Millipore filter (0.45 μm), washed three times with 2 ml of a solution containing 100 mM KCl and 20 mM imidazole (pH 7.0), and counted in a liquid scintillation counter. For each experiment, a blank filter was treated the same way with assay medium but not containing SR. The radioactivity in the blank filter was subtracted from the experimental values.

Determination of partition coefficients

The systems used for the determination of partition coefficients were: (a) oil/Ringer's solution (1:1), (b) octanol/Ringer's solution (1:1), and (c) chloroform/Ringer's solution (1:1). [Corn oil (Crisco brand) was used as the oil.] All measurements were performed at room temperature. One ml of Ringer's solution, containing 10^{-7} M or 10^{-6} M calcium antagonist and trace amounts of tritiated drug (1 μCi/ml), was mixed vigorously with 1 ml of either oil, octanol, or chloroform for 5 min with a Virtex mixer. The samples were then centrifuged at 2000 *g* for 5 min to separate the phases. A 0.5 ml sample of the upper phase was pipetted into scintillation vials for counting, and the remainder of the upper phase was aspirated and discarded. Then 0.5 ml of the lower phase was pipetted into scintillation vials for counting. Quenching of radioactivity by chloroform in the scintillation mixture was corrected for before calculation of the partition coefficients. Partition coefficient is defined as the ratio of the amount of radioactive calcium antagonistic drug present in the organic phase to the amount of radioactive drug in the aqueous phase (Ringer's solution).

Calcium antagonistic drugs

Nifedipine was obtained from Pfizer Pharmaceuticals, diltiazem from Marion Laboratory, bepridil from Wallace Laboratories, verapamil–HCl from Knoll Pharmaceuticals, and nitrendipine from Miles Institute for Preclinical Pharmacology. [^{3}H] Nitrendipine (88 Ci/mmole) was obtained from the New England Nuclear Corp. The other four calcium antagonists were tritiated by ICN Pharmaceuticals, Inc., using silent electric discharge. The specific radioactivities of the randomly labeled compounds were in the range of 20–30 Ci/mmole. The structures of the drugs used are shown in Fig. 1.

RESULTS

Uptake of calcium antagonistic drugs into rat skeletal muscle

Figure 2 shows the time courses of uptake of calcium antagonistic drugs into rat skeletal muscle. The uptake values of the drugs, especially nitrendipine, nifedipine and diltiazem, reached maxima at 80 min. At equilibrium, the influx of the labeled drug should be equal to the efflux of labeled drug. The uptake of bepridil was highest, and reached a value of 2.43 ± 0.22 pmoles/mg protein at 120 min (Fig. 2 and Table 1). The order of maximum uptake values was: bepridil > nitrendipine ≥ verapamil > nifedipine > diltiazem (Table 1). The relative amounts of drugs taken up by the skeletal muscle could be deduced from the T/M ratio, an indicator of the distribution of the drugs in the muscle at steady state with respect to that in the medium (Table 1).

Binding of calcium antagonistic drugs to isolated skeletal sarcoplasmic reticulum

Table 2 shows that calcium antagonists were bound to, or were taken up into, the isolated skeletal sarcoplasmic reticulum (SR). (It is not possible to distinguish between binding and uptake into the lumen of the vesicles in these experiments.) The order of uptake was: verapamil > nitrendipine > bepridil > nifedipine > diltiazem.

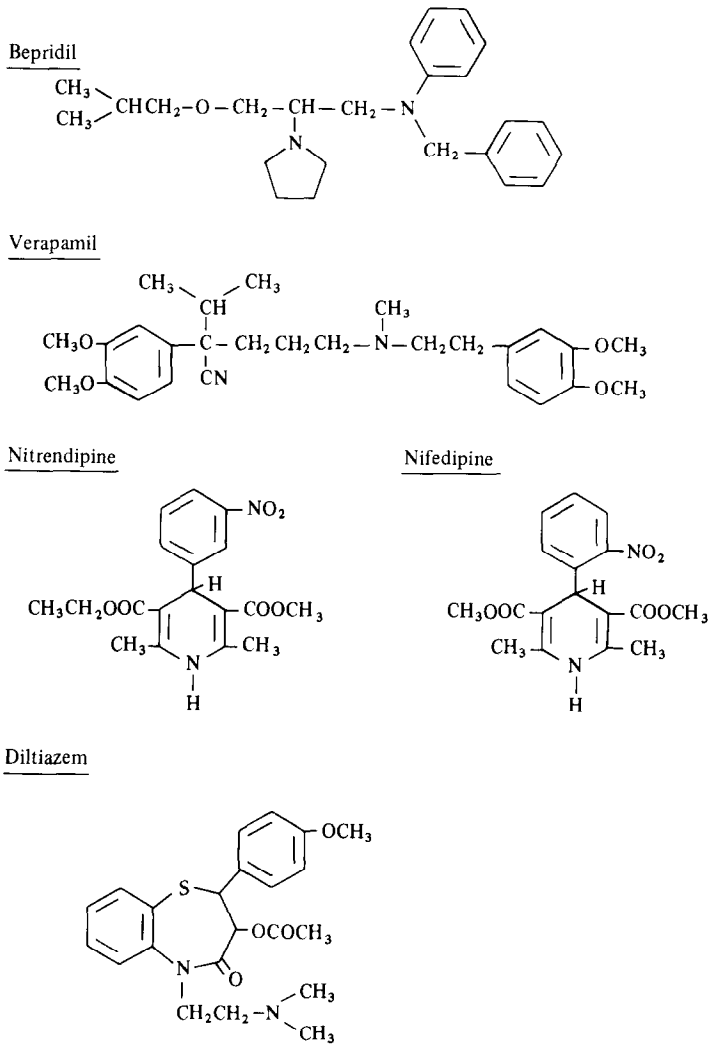

Fig. 1. Structural formulae of the calcium antagonistic drugs used in the present study.

Uptake of calcium antagonists into monolayer cultures of vascular smooth muscle cells from rat aorta

To determine whether the calcium antagonistic drugs were bound to the surface membrane and/or were accumulated inside the muscle cells, the uptake values of the drugs into monolayer cultures of vascular muscle cells from rat aorta were measured (Table 3). Since, after incubation, the monolayer cultures were washed with 10^{-5} M cold (unlabeled) drug to displace surface binding, drug uptake in these experiments should represent internal accumulation. Table 3 shows that bepridil, nitrendipine and verapamil were all accumulated by the vascular smooth muscle cells, whereas nifedipine and diltiazem were not. The order of uptake was: bepridil > nitrendipine > verapamil ≫ nifedipine = diltiazem, which was similar to that found in rat skeletal muscle (Table 1).

Uptake of verapamil and bepridil into glycerol-treated frog sartorius muscle

To determine the possible site of entry of the calcium antagonistic drugs, bepridil and verapamil, into skeletal muscle fibers, frog sartorius muscle was treated with glycerol to disrupt the T-tubules. Uptake of either bepridil or verapamil into frog sartorius muscle was not affected by glycerol treatment (Fig. 3). The uptake of bepridil was higher than that of verapamil, as was found for the rat EDL muscle (Fig. 2) and for cardiac muscle and smooth muscle [6].

Partition coefficient of calcium antagonistic drugs

The partition coefficients of these agents in oil/Ringer's solution, octanol/Ringer's solution, and chloroform/Ringer's solution were measured. Table 4 summarizes these data. The order of the partition coefficients for the drugs in oil and octanol was: bepridil > verapamil > nitrendipine > nifedipine ≫ diltiazem. The order was slightly different for the partition coefficients in the chloroform/Ringer's solution system: verapamil > bepridil ≫ nitrendipine > nifedipine ≫ diltiazem. Thus, in oil and octanol systems, the order of the partition coefficients for the drugs was similar to the order of the values for

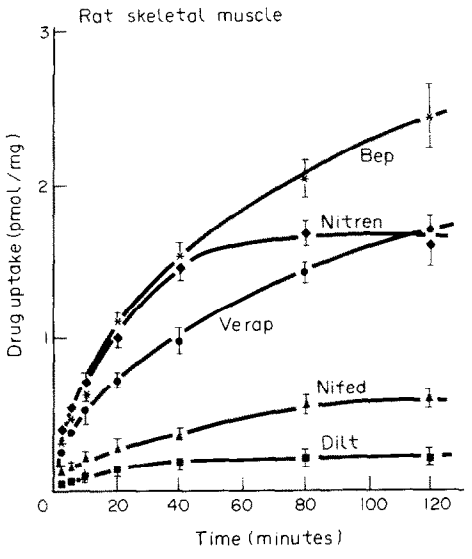

Fig. 2. Time courses of uptake of calcium antagonists into rat skeletal muscle (extensor digitorum longus). Uptake of each calcium antagonist was measured in the presence of 145 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10^{-7} M [^{3}H]calcium antagonist, 20 mM HEPES, pH 7.4, and 10 mM glucose at 37°. Values are expressed as mean ± S.E.M.

uptake of these drugs into the skeletal muscles (rat and frog) and into monolayer cultures of vascular smooth muscle cells (rat).

DISCUSSION

The present study indicates that, of the five calcium antagonists tested, bepridil, verapamil and nitrendipine were taken up by the intact skeletal muscle fibers. The amount of calcium antagonists taken up by the muscle represents internal accumulation, as well as some possible residual binding to the surface membrane [6, 7]. The present data from monolayer cultures of vascular smooth muscle cells also suggest that nifedipine and diltiazem did not enter the cells (Table 3), whereas bepridil, nitrendipine and verapamil did. Accumulation of the latter three drugs (probably by passive diffusion [7]) is also supported by the high calculated $[Drug]_i/[Drug]_o$ ratio (Table 1).

Table 2. Uptake of calcium antagonists in isolated sarcoplasmic reticulum (SR) from rat skeletal muscles (hind limb)*

	Drug binding (pmoles/mg protein)
Verapamil	34.9 ± 0.0
Nitrendipine	19.9 ± 4.5
Bepridil	15.7 ± 1.9
Nifedipine	10.7 ± 1.5
Diltiazem	2.1 ± 0.2

* SR was incubated in 100 mM KCl, 20 mM imidazole (pH 7.0), and 10^{-7} M drug at room temperature for 10 min. SR was then collected by Millipore filtration. Data are expressed as mean ± S.E.M. from five experiments.

The present study also shows that the calcium antagonists did not have to enter the skeletal muscle fibers through the T-tubules, because disconnection of these structures from the surface membrane in the frog muscle through glycerol treatment did not decrease the amount of bepridil and verapamil taken up by the frog sartorius muscle (Fig. 3). Kerr and Sperelakis [3] have shown that the slow action potentials, which are induced in frog skeletal muscle fibers by bathing them in Cl^--free solution, disappear in detubulated fibers, thus indicating that the preponderance of the Ca^{2+} slow channels are located in the T-tubular system. Therefore, this suggests that the entrance of the drugs into the fibers was not through the slow channels themselves but rather through the lipid bilayer matrix of the cell membrane, in accordance with the lipid solubility of the drug.

One possible mechanism of intracellular accumulation of calcium antagonists is by binding of these agents to internal sites, such as the SR and myofilaments. The present data indicate that bepridil, verapamil, nitrendipine, and nifedipine became bound to isolated SR membrane and were taken up into the intravesicular lumen (Table 2). Interestingly,

Table 1. Characteristics of uptake of calcium antagonists into rat skeletal muscle (EDL)*

	Drug uptake				
	Initial rates (pmoles/mg/min)	$T_{\frac{1}{2}}$ (min)	Maximal uptake (pmoles/mg protein)	T/M ratio	$\frac{[Drug]_i}{[Drug]_o}$
Bepridil	0.14 ± 0.00	25	2.43 ± 0.22	3.51	5.49
Nitrendipine	0.16 ± 0.01	13	1.62 ± 0.14	2.34	3.66
Verapamil	0.11 ± 0.01	27	1.57 ± 0.10	2.27	3.55
Nifedipine	0.05 ± 0.01	22	0.59 ± 0.04	0.85	1.33
Diltiazem	0.03 ± 0.00	10	0.23 ± 0.02	0.33	0.52

* Initial rates were calculated from values obtained at 2.5 min in the uptake curves. Maximal uptake was calculated from values at 80 and 120 min. $T_{\frac{1}{2}}$ was obtained graphically. T/M ratio is expressed as pmoles per g wet wt/pmoles per ml of medium $[Drug]_i/[Drug]_o$ was calculated from the T/M ratio after correction (as described in text). Data are expressed as mean ± S.E.M. from six experiments. Incubation of extensor digitorum longus muscle was carried out at 37° in Ringer's solution containing 10^{-7} M drug.

Table 3. Uptake of calcium antagonists into monolayer cultures of vascular smooth muscle cells of rat aorta*

	Concn (M)	Drug uptake (pmoles/mg protein)
Bepridil	10^{-7}	9.23 ± 0.81
	10^{-6}	90.3 ± 4.8
Nitrendipine	10^{-7}	5.32 ± 0.32
	10^{-6}	43.3 ± 3.5
Verapamil	10^{-7}	3.23 ± 0.70
	10^{-6}	33.2 ± 4.2
Nifedipine	10^{-7}	ND†
	10^{-6}	ND
Diltiazem	10^{-7}	ND
	10^{-6}	ND

* Incubation of monolayer cultures was carried out at 37° for 20 min in Ringer's solution containing the drugs. The cultures were then washed three times with Ringer's solution containing 10^{-5} M unlabeled drug. Data are expressed as mean ± S.E.M. from six experiments. For nifedipine and diltiazem, the amounts of drugs taken up by the cultures after 20 min incubation were the same as with exposure for 2 sec only, i.e. there was no detectable uptake.
† Not detectable.

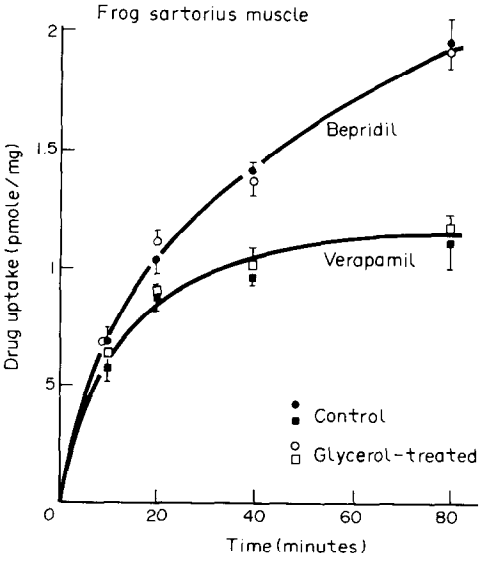

Fig. 3. Effect of detubulation on the uptake of [^{3}H]bepridil and [^{3}H]verapamil into frog sartorius muscle. Detubulation was accomplished by sudden immersion in glycerol-free Ringer's solution after incubation in 300 mM glycerol/Ringer's solution for 60 min. Uptake of calcium antagonist was measured in the presence of 133 mM NaCl, 1.88 mM KCl, 1.08 mM $CaCl_2$, 10^{-7} M ^{3}H-drug, 20 mM HEPES, pH 7.4, and 10 mM glucose at room temperature. Values are expressed as mean ± S.E.M. (four experiments).

verapamil binding/uptake to SR was higher than nitrendipine and bepridil, contrary to the order of the values for uptake of these drugs in the intact muscle (Table 1). It is likely that the drugs were bound to a variety of internal binding sites. For example, nitrendipine binds to isolated cardiac sarcolemma, sarcoplasmic reticulum and mitochondrial membranes [12], and bepridil binds to cardiac actin [13].

The concentrations of calcium antagonists (10^{-7}–10^{-6} M) tested in the present study are comparable to that observed clinically in the plasma (~10^{-7} M) and that achieved in the human [14] and canine [15] myocardium (~10^{-6} M). Although the muscles are able to accumulate the calcium antagonists (Table 1, [6, 7]), the internal concentrations of these agents were still below that observed (10^{-4} M) to be effective in stimulating or depressing calcium uptake by the isolated sarcoplasmic reticulum [16, 17].

The ability of calcium antagonists to enter and react with multiple internal sites correlates well with published functional studies showing that some of these drugs may exert a second effect on internal sites [4, 5]. In addition, Hescheler *et al.* [18] recently showed that a derivative of verapamil, D-890, which could not cross the surface membrane because of its structure, blocked the slow inward Ca^{2+} current only after microinjection inside the cardiac cells, suggesting that verapamil and its derivatives may act on internal sites of the surface membrane.

The lipid solubility of each of the five calcium antagonists tested was related to its ability to enter the muscle cells (Tables 1 and 4). This suggests that the drugs permeate the cell membrane by solubilizing

Table 4. Partition coefficients of calcium antagonists*

	Concn (M)	Partition coefficients		
		Oil/Ringer	Octanol/Ringer	Chloroform/Ringer
Bepridil	10^{-7}	30.9 ± 1.4	102.0 ± 4.3	30.1 ± 1.0
	10^{-6}	32.9 ± 3.1	98.7 ± 5.3	30.1 ± 1.4
Verapamil-HCl	10^{-7}	16.7 ± 0.3	67.8 ± 0.3	39.2 ± 3.1
	10^{-6}	17.0 ± 0.5	66.6 ± 0.5	40.2 ± 1.1
Nitrendipine	10^{-7}	7.92 ± 0.09	9.28 ± 0.36	5.26 ± 0.61
	10^{-6}	8.16 ± 0.05	9.95 ± 0.25	5.11 ± 0.32
Nifedipine	10^{-7}	2.33 ± 0.02	2.68 ± 0.04	2.19 ± 0.18
	10^{-6}	2.30 ± 0.03	2.64 ± 0.02	2.28 ± 0.18
Diltiazem	10^{-7}	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.00
	10^{-6}	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.00

* Measurements were made at room temperature with three different lipid solvents (1 part lipid solvent to 1 part Ringer solution). Data are expressed as mean ± S.E.M. from six experiments, except in the case of bepridil and verapamil for the chloroform experiments, in which eighteen experiments were performed. The oil used was corn oil (Crisco brand).

in the lipid bilayer matrix. As expected, there was no correlation between the oil/water partition coefficients of the calcium antagonists and their abilities to block the Ca^{2+} slow channels, as indicated by inhibition of the slow inward current (I_{si}) and Ca^{2+} uptake into intestinal smooth muscle.

There are four classes of structures among the calcium antagonistic drugs studied. Verapamil, diltiazem, bepridil and both 1,4-dihydropyridines (nitrendipine and nifedipine) all have markedly different molecular structures (Fig. 1). Nitrendipine (IC_{50} of 3.1×10^{-9} M) is more potent for inhibition of K^+-induced contractions of isolated rabbit aortic smooth muscle than nifedipine (IC_{50} of 8.1×10^{-9} M) [19]. Nitrendipine stimulates Ca^{2+} sequestration by both cardiac and skeletal SR, depending on the drug/membrane phospholipid mole ratios, and the authors suggested that the drug interacts with the membrane phospholipids; nifedipine has no such effect [20]. Verapamil (1.7×10^{-7} M) did not inhibit Ca^{2+} uptake in the isolated SR. There is a correlation between the partition coefficients and anti-calmodulin potencies of phenothiazines and related drugs (a series of ring-substituted promazine derivatives) on the calmodulin-stimulated phosphodiesterase activity [21].

In summary, the present study shows that verapamil, bepridil, and nitrendipine entered and accumulated inside the skeletal muscle fibers, whereas nifedipine and diltiazem did not readily permeate. The permeation of the drugs through the cell membrane was correlated with their respective lipid solubilities.

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REFERENCES

1. K. Shigenobu, J. A. Schneider and N. Sperelakis, *J. Pharmac. exp. Ther.* **190**, 280 (1974).
2. D. R. Harder and N. Sperelakis, *J. cardiovasc. Pharmac.* **3**, 906 (1981).
3. L. M. Kerr and N. Sperelakis, *J. Pharmac. exp. Ther.* **222**, 80 (1982).
4. S. Vogel, R. Crampton and N. Sperelakis, *J. Pharmac. exp. Ther.* **210**, 378 (1979).
5. S. Mras and N. Sperelakis, *Blood Vessels* **18**, 196 (1981).
6. D. C. Pang and N. Sperelakis, *Eur. J. Pharmac.* **87**, 199 (1983).
7. D. C. Pang and N. Sperelakis, *Biochem. Pharmac.* **32**, 1660 (1983).
8. D. R. Harder and N. Sperelakis, *Blood Vessels* **16**, 186 (1979).
9. D. C. Pang and N. Sperelakis, *J. molec. cell. Cardiol.* **14**, 703 (1982).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 365 (1951).
11. D. C. Pang and F. N. Briggs, *J. biol. Chem.* **252**, 3262 (1977).
12. J. G. Sarmiento, R. A. Janis, R. A. Colvin, D. J. Triggle and A. M. Katz, *J. molec. cell. Cardiol.* **15**, 135 (1983).
13. G. Cramb and J. W. Dow, *Biochem. Pharmac.* **32**, 227 (1983).
14. R. E. Kates, D. Keefe, J. Schwartz, S. Harapat, D. Harrison, E. Kirsten and S. M. Johnson, *Clin. Pharmac. Ther.* **29**, 257 (1981).
15. D. L. Keefe and R. E. Kates, *J. Pharmac. exp. Ther.* **220**, 91 (1982).
16. R. A. Colvin, N. Pearson, F. C. Messineo and A. M. Katz, *J. cardiovasc. Pharmac.* **4**, 935 (1982).
17. M. L. Entman, J. C. Allen, E. P. Bornet, P. C. Gillette, E. T. Wallick and A. Schwartz, *J. molec. cell. Cardiol.* **4**, 681 (1972).
18. J. Hescheler, D. Pelzer, G. Trube and W. Trautwein, *Pflügers Archs* **393**, 287 (1982).
19. R. Towart, *J. cardiovasc. Pharmac.* **4**, 895 (1982).
20. R. A. Colvin, N. Pearson, F. C. Messineo and A. M. Katz, *J. cardiovasc. Pharmac.* **4**, 935 (1982).
21. W. C. Prozialeck and B. Weiss, *J. Pharmac. exp. Ther.* **222**, 509 (1982).