1,4-Dihydropyridine Antagonist Activities at the Calcium Channel: A Quantitative Structure–Activity Relationship Approach

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The effect of 46 1,4-dihydropyridine-type calcium channel antagonists on the tonic contractile response of longitudinal muscle strips of guinea pig ileum was determined. 2,6-Dimethyl-3,5-dicarbomethoxy-4-phenyl-1,4-dihydropyridine (13) and 13 ortho-, 15 meta-, and seven para-monosubstituted and 10 polysubstituted aromatic derivatives of 13 were studied. The pharmacological activities of the monosubstituted derivatives were best correlated by eq 10, log $1/C = 0.68\pi + 2.50\sigma_m - 0.47L_{meta} - 3.40B_{1_{para}} + 11.31$, which had a correlation coefficient of 0.89. The full data set was best correlated by eq 11, log $1/C = 0.62\pi + 1.96\sigma_m - 0.44L_{meta} - 3.26B_{1_{para}} - 1.51L_{meta'} + 14.23$, which had a correlation coefficient of 0.90. Equations of similar form but involving an ortho steric term were found to correlate the radioligand binding data for this class of compounds.

The Ca^{2+} channel antagonists, including the clinically available verapamil, diltiazem, and nifedipine, are widely used in the treatment of a number of cardiovascular disorders[1] and offer considerable potential in other areas including nonvascular smooth muscle and neuronal disorders.[2] The 1,4-dihydropyridine class, represented by nifedipine, includes the most potent Ca^{2+} channel antagonists (and activators) and because of the relative ease of synthesis has been the object of a number of structure–activity studies.[2-12] These studies, both qualitative and quantitative, have been based on in vivo and in vitro pharmacologic determinations and upon radioligand binding measurements.[2]

Solid-state structural studies have suggested the importance of a conformation in which the 4-aryl ring of 2,6-dimethyl-3,5-dicarboalkoxy-4-aryl-1,4-dihydropyridines is pseudoaxial and oriented in perpendicular fashion over the 1,4-dihydropyridine ring which is in a flattened boat conformation.[3,4] The syntheses and activities of rigid analogues lend support to this conclusion.[5,6]

Aryl ring substituents exert significant effects both on binding and on pharmacological activity. Para substitution in the 4-phenyl ring leads to activity loss regardless of substituent type.[2,7,8] Rodenkirchen et al.[9,10] evaluated the pharmacological effect of 14 nifedipine derivatives consisting of seven ortho-, four meta-, and two para-monosubstituted compounds and the phenyl-unsubstituted compound on cardiac muscle. Rodenkirchen noted an overall correlation of lipophilicity to biological activity but put forward only one quantitative relationship, namely that the biological potency of eight ortho-substituted analogues correlates with the Verloop sterimol parameter B_1 (eq 1).

$$\log 1/EC_{50} = 5.06 + 0.80B_1 \quad (1)$$

$$n = 8,\ r = 0.91,\ s = 0.12$$

Recently Mahmoudian and Richards[11] studied the quantitative structure–activity relationship (QSAR) of binding of nifedipine analogues to guinea pig ileal preparations as determined by Bolger et al.[8] Richards drew the following conclusions.

1. Binding of ortho-substituted compounds correlates positively with B_1, but does not correlate significantly with σ, π, or L.

2. Meta-substituted compounds correlate directly with σ_m and the correlation can be improved slightly by addition of terms based on π, B_1, or L. The correlations with π and B_1 are direct and that with L is inverse.

3. Para-substituted compounds were found to correlate inversely with L or B_1 and the correlation could be improved by addition of an L^2 or B_1^2 term.

4. The "complete set" of data for 18 compounds could be correlated ($r = 0.67$) by eq 2:

$$\log 1/IC_{50} = 8.491 + 0.636\pi_{ortho} - 3.146\pi_{para} \quad (2)$$

5. The "complete set" of data is best correlated by the eq 3 or 4:

$$\log 1/IC_{50} = 7.566 + 2.238B_{1_{o,m}} - 0.479L_m - 1.288B_{1_p} + 1.948\sigma_m$$

$$r = 0.93 \quad (3)$$

$$\log 1/IC_{50} = 7.430 + 2.376B_{1_{o,m}} - 0.472L_m - 0.674L_p + 1.928\sigma_m$$

$$r = 0.93 \quad (4)$$

In a study[12] involving binding data on only seven compounds, Berntsson and Wold concluded that "electron demanding substituents on the ring enhance activity, while bulk in the para position decreases activity".

Given the limited number of compounds upon which previous studies have been based and the divergent behavior of ortho, meta, and para derivatives, we have reexamined these QSAR studies by substantially increasing the data base in an effort to refine the factors contributing to both binding and pharmacological activity.

(1) For review, see: Triggle, D. J.; Janis, R. A. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 347 and references cited therein.
(2) Janis, R. A.; Silver, P.; Triggle, D. J. *Adv. Drug Res.* **1987**, *16*, 309.
(3) Triggle, A. M.; Shefter, E.; Triggle, D. J. *J. Med. Chem.* **1980**, *23*, 1442.
(4) Fossheim, R.; Svarteng, K.; Mostad, A.; Romming, C.; Shefter, E.; Triggle, D. J. *J. Med. Chem.* **1982**, *25*, 126.
(5) Seidel, W.; Meyer, H.; Born, L.; Kazda, S.; Dompert, W. In *Abstracts of Papers*, 187th National Meeting of the American Chemical Societ, St. Louis, MO, April 8–13, 1984; American Chemical Society: Washington, DC, 1984.
(6) Baldwin, J. J.; Claremon, D. A.; Lumma, P. K.; McClure, D. E.; Rosenthal, S. A.; Linquist, R. J.; Faison, E. P.; Kaczorowski, G. J.; Trumble, M. J.; Smith, G. M. *J. Med. Chem.* **1987**, *30*, 690.
(7) Loev, B.; Goodman, M. M.; Snader, K. M.; Tedeschi, R.; Macko, E. *J. Med. Chem.* **1974**, *17*, 956.
(8) Bolger, G. T.; Gengo, P.; Klockowski, R.; Luchowski, E.; Siegel, H.; Janis, R. A.; Triggle, A. M.; Triggle, D. J. *J. Pharmacol. Exp. Ther.* **1983**, *225*, 291.
(9) Rodenkirchen, R.; Bayer, R.; Steiner, F.; Bossert, H.; Meyer, H.; Moller, E. *Naunyn. Schmiedeberg's Arch. Pharmacol.* **1979**, *310*, 69.
(10) Mannhold, R.; Rodenkirchen, R.; Bayer, R. *Prog. Pharmacol.* **1982**, *5*, 25.
(11) Mahmoudian, M.; Richards, G. W. *J. Pharm. Pharmacol.* **1986**, *38*, 272.
(12) Berntsson, P.; Wold, S. *Quant. Struct.-Act. Relat. Pharmacol., Chem., Biol.* **1986**, *5*, 45.

0022-2623/88/1831-2103$01.50/0

Discussion

A careful reading of the report by Richards[11] reveals a number of peculiarities including the following.

1. The σ values used for substituents at position 2 are clearly and correctly labeled by Norrington[13] as derived from phenols. As such, they are subject to "through resonance" and are of uncertain relevance to the case under discussion.

2. The physical parameters π and σ are claimed to be taken from Norrington,[13] but in contrast to Richards, the Norrington reference gives no π value for 2-NO_2. There are small discrepancies between the values quoted in the two papers for π for 2-CH_3 and π for 4-CH_3.

3. Many of the π values in Norrington are taken from an earlier study by Hansch on phenoxyacetic acids. The more recent and frequently different values given by Hansch[14] seem to be a more appropriate data set.

4. Without so noting or explaining, Richards deletes one monosubstituted compound (3-N^+Me_3) and two polysubstituted compounds from Triggle's original set of binding data.[8]

5. The largest number of compounds in Richards' data set are meta. In spite of this, eq 2, which purports to correlate the entire data set, contains no physical parameter related to the meta substituents.

6. Equations 3 and 4 are inconsistent as written because the value for hydrogen is substituted, when appropriate, in the last three terms but is not substituted in the second term for ortho- or meta-substituted compounds. We presume that what was intended is

$$\log 1/IC_{50} = 5.328 + 2.238(B_{1_o} + B_{1_m}) - 0.479L_m - 1.288B_{1_p} + 1.948\sigma_m \quad (5)$$

$$\log 1/IC_{50} = 5.054 + 2.376(B_{1_o} + B_{1_m}) - 0.472L_m - 0.074L_p + 1.928\sigma_m \quad (6)$$

where B_{1_o} = sterimol B_1 for the ortho substituent, B_{1_m} = sterimol B_1 for the meta substituent, and L_m = sterimol L for the meta substituent.

In addition to the inconsistencies noted above, all previous studies represent small data sets and are difficult to compare because they were derived from tension measurements in cardiac muscle[9] and radioligand binding in smooth muscle.[8] Because of the voltage dependence of 1,4-dihydropyridine interactions at Ca^{2+} channels, these preparations do not reflect the same quantitative expression of activities.[1,15] Additionally, the opposing activator and antagonist properties of some stereoisomeric pairs of 1,4-dihydropyridines may complicate the interpretation of the activities of racemic compounds.[16,17]

These considerations suggested the value of reanalyzing some earlier data and of analyzing a larger set of nonchiral 1,4-dihydropyridine antagonists derived from 2,6-dimethyl-3,5-dicarbomethoxy-4-phenyl-1,4-dihydropyridine bearing ortho, meta, para, and multiple substituents in the phenyl ring and where the biological activities were determined in a single preparation. In a re-investigation of published work, we began by using eq 7, reported by Richards' for the subset of ortho-substituted compounds to calculate the $-\log IC_{50}$ for binding of the ortho iodo and ortho bromo compounds. These two compounds were not included in Triggle's original data set. In both cases the calculated values deviated from the observed values by more than the reported standard deviation for eq 7. However, regression of the data set for all nine ortho-substituted compounds gave eq 8, which is similar in form to eq 7 and has a better statistical fit. It may be demonstrated, in this fashion, that all the equations reported by Richards, except for eq 2, can accommodate these two additional data points with only minor adjustments.

$$\log 1/IC_{50} = 5.152\ (\pm 0.74) + 2.407\ (\pm 0.49)B_{1_{ortho}}$$
$$n = 7,\ r = 0.91,\ s = 0.32 \quad (7)$$

$$\log 1/IC_{50} = 4.48\ (\pm 0.56) + 2.91\ (\pm 0.34)B_{1_{ortho}}$$
$$n = 9,\ r = 0.96,\ s = 0.33,\ F = 73.07 \quad (8)$$

Equation 2 obviously calculates different means for the subsets of data corresponding to the ortho-, meta-, and para-substituted compounds. It appears to be statistically valid for Richards' selected set of compounds only because the range of values about the mean for each of the subsets is small. However, the 2-iodo and 2-bromo compounds both bind sufficiently more tightly than any of the related compounds to invalidate both eq 2 and the arguments based on eq 2 which were used to support a model for the binding site. The 3-N^+Me_3 compound which Richards deleted from the original data set also fails to fit eq 2.

We then attempted to correlate the tonic components of the guinea pig ileum tension response to muscarinic receptor stimulation to physical parameters for a set of 46 nifedipine analogues. The set varied only in substitution on the phenyl ring and consisted of one unsubstituted, 13 ortho-monosubstituted, 15 meta-monosubstituted, seven para-monosubstituted, and ten polysubstituted compounds as shown in Table I. This is the largest set of dihydropyridine-type calcium channel antagonists thus far subjected to Hansch-type regression analysis. The greater size of the data base permitted us to check the validity of earlier correlations and to investigate more complex relationships than those previously considered.

In contrast to previous studies, we found little correlation between B_1 of ortho-monosubstituted compounds and pharmacologically activity ($r = 0.44$). Numerous attempts were made to correlate the data for all monosubstituted compounds or that for the ortho- and meta-substituted subset by use of equations containing up to three parameters with not more than one parameter being steric. Most correlations were very poor, and none was as good as $r = 0.85$.

The remainder of our work was based on the following assumptions. Because steric interactions are by their nature localized, each position on the aromatic ring might require a separate term to adequately describe its steric interactions. We assume that for ortho- and meta-monosubstituted compounds the aromatic ring always has the same conformation and, to the extent that steric factors prove important, that the steric contributions for the normally unoccupied ortho' and meta' positions are less favorable than those for the ortho and meta positions. Conversely, if any position on the aromatic ring is sensitive to the electronic properties of substituents, we would expect all positions to be equally sensitive (although in the case of interaction with a dipole on the receptor, vector analysis is required and vectors orthogonal to the dipole may be disregarded). To limit the number of terms, we have chosen to consider only the net effect of all aromatic

(13) Norrington, F. E.; Hyde, R. M.; Williams, S. G.; Wooton, R. *J. Med. Chem.* **1975**, *18*, 604.
(14) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979.
(15) Chin, J. H. *Biochem. Pharmacol.* **1986**, *35*, 4115.
(16) Hof, R. P.; Ruegg, V. T.; Hof, A.; Vogel, A. *J. Cardiovas. Pharmacol.* **1985**, *7*, 689.
(17) Wei, X. Y.; Luchowski, E. M.; Rutledge, A.; Su, C. M.; Triggle, D. J. *J. Pharmacol. Exp. Ther.* **1986**, *239*, 144.

Table I. Parameters for the Dihydropyridines Used in These QSAR Studies

no.	X	log 1/C obsd[a]	tonic calcd[b]	response calcd[c]	log 1/C obsd[d]	binding calcd[e]	π	σ_m	B_1	L
1	3-Br	8.89	7.48	7.65			0.86	0.39	1.95	3.83
2	2-CF_3	8.82	8.35	8.61			0.88	0.43	1.98	3.30
3	2-Cl	8.66	8.13	8.34	9.78	9.87	0.71	0.37	1.80	3.52
4	3-NO_2	8.40	7.57	7.85	9.97	9.08	−0.28	0.71	1.70	3.44
5	2-CH=CH_2	8.35	7.57	7.62			0.82	0.05	1.60	4.29
6	2-NO_2	8.29	8.18	8.51	9.08	9.77	−0.28	0.71	1.70	3.44
7	2-CH_3	8.22	7.17	7.14	8.71	8.43	0.56	−0.07	1.52	3.00
8	2-Et	8.19	7.45	7.45			1.02	−0.07	1.52	4.11
9	2-Br	8.12	8.26	8.49	10.49	10.18	0.86	0.39	1.95	3.83
10	2-CN	7.80	7.71	7.94	9.18	9.08	−0.57	0.56	1.60	4.23
11	3-Cl	7.80	7.48	7.64	9.30	9.08	0.71	0.37	1.80	3.52
12	3-F	7.68	7.46	7.59	8.49	8.55	0.14	0.34	1.35	2.65
13	H	7.55	6.96	6.93	7.85	7.63	0	0	1.00	2.06
14	3-CN	7.46	6.75	6.90	8.68	8.49	−0.57	0.56	1.60	4.23
15	3-I	7.38	7.38	7.53			1.12	0.35	2.15	4.23
16	2-F	7.37	7.72	7.88	8.44	8.89	0.14	0.34	1.35	2.65
17	2-I	7.33	8.34	8.57	10.80	10.50	1.12	0.35	2.15	4.23
18	2-OCH_3	7.24	7.19	7.22	7.87	8.24	−0.02	0.12	1.35	3.98
19	3-CF_3	7.13	7.80	8.01			0.88	0.43	1.98	3.30
20	3-CH_3	6.96	6.76	6.69	7.28	7.92	0.56	−0.07	1.52	3.00
21	2-OEt	6.96	7.39	7.44			0.38	0.10	1.35	4.92
22	3-OCH_3	6.72	6.34	6.30	7.27	7.90	−0.02	0.12	1.35	3.98
23	3-N Me_2	6.05	6.13	5.98			0.18	−0.15	1.50	3.53
24	3-OH	6.00	6.49	6.45			−0.67	0.12	1.35	2.74
25	3-NH_2	5.70	5.51	5.28			−1.23	−0.16	1.50	2.93
26	3-OAc	5.22	6.09	6.12			−0.64	0.39	1.35	4.87
27	3-O-COPh	5.20	5.59	5.54			1.46	0.21	1.70	8.15
28	2-NH_2	4.40	5.89	5.69			−1.23	−0.16	1.50	2.93
29	3-N^+Me^3	4.30	4.15	4.13	<5.00	4.89	−5.96	0.88	2.56	4.02
30	4-F	6.89	6.58	6.69	7.46	7.43	0.14	0.34	1.35	2.65
31	4-Br	5.40	5.16	5.27			0.86	0.39	1.95	3.83
32	4-I	4.64	4.59	4.66			1.12	0.35	2.15	4.23
33	4-NO_2	5.50	5.90	6.14	6.52	6.85	−0.28	0.71	1.70	3.44
34	4-NMe_2	4.00	5.15	4.98			0.18	−0.15	1.50	3.53
35	4-CN	5.46	4.26	4.34			−0.57	0.56	2.06	4.23
36	4-Cl	5.09	5.52	5.62	6.22	6.56	0.71	0.37	1.80	3.52
37	2,6-Cl_2	8.72	9.29				1.42	0.74		
38	F_5	8.36	8.44		10.37		0.70	1.70		
39	2-F, 6-Cl	8.12	8.88		8.37		0.85	0.71		
40	2,3-Cl_2	7.72	8.65				1.42	0.74		
41	2-Cl, 5-NO_2	7.52	7.27				0.43	1.08		
42	3,5-Cl_2	7.03	6.45				1.42	0.74		
43	2-OH, 5-NO_2	7.00	7.40				−0.95	0.83		
44	2,5-Me_2	7.00	6.96				1.12	−0.14		
45	2,4-Cl_2	6.40	6.68				1.42	0.74		
46	2,4,5-$(OCH_3)_3$	3.00	3.60				−0.06	0.36		
47	3-N_3				8.67	8.64	0.46	0.27	1.50	4.62
48	4-CH_3				7.18	6.27	0.56	−0.07	1.52	3.00

[a] Where C is the molar EC_{50} value required to cause the response described under Pharmacology. [b] Calculated by eq 11. [c] Calculated by eq 10. [d] Where C is the IC_{50} value measured is described in ref 8. [e] Calculated by eq 12.

Table II. Pairwise Correlation Matrix for Data Used in Eq 11

	log 1/C	π	σ_m	L_o	L_m	L_p
log 1/C	1.000					
π	0.391	1.000				
σ_m	0.224	−0.068	1.000			
B_{1o}	0.443	0.281	−0.213	1.000		
L_m	−0.135	−0.072	0.014	−0.435	1.000	
B_{1p}	−0.500	0.100	0.151	−0.210	−0.295	1.00

substituents on the lipophilicity of the molecule and not to look for position-specific terms based on π.

We considered first only the data on the unsubstituted compound plus the ortho- and meta-monosubstituted compounds. Equations containing up to four variables—one steric term for ortho substituents, one for meta, π, and an electronic term—were investigated; the best equation found was eq 9 in which the appropriate value of σ_m is

$$\log 1/IC_{50} = 0.69\ (\pm 0.10)\pi + 2.32\ (\pm 0.49)\sigma_m - 0.49(\pm 0.10)L_{meta} + 8.01\ (\pm 0.32) \quad (9)$$

$$n = 29,\ r = 0.87,\ s = 0.67,\ F = 25.35$$

entered for the substituents regardless of whether it is in the 2- or 3-position. The inclusion of either an L term or B_1 term for ortho substituents did not result in a significant improvement. The multiple correlation coefficient was 0.88 for these four-term equations.

Addition of the seven para-substituted compounds to the data set gave eq 10. Exclusion of the para steric term

$$\log 1/IC_{50} = 0.68\ (\pm 0.10)\pi + 2.50\ (\pm 0.45)\sigma_m - 0.47(\pm 0.10)L_{meta} - 3.40\ (\pm 0.37)B_{1_{para}} + 11.31\ (\pm 0.55) \quad (10)$$

$$n = 36,\ r = 0.89,\ s = 0.68,\ F = 30.46$$

Table III. Physical Properties of Prepared Compounds

no.	Ar–X	mp, °C	lit. mp, °C	yield, %	solvent	analyses[a]
1	3-Br	196–198		79	MeOH	C,[b] H, N
2	2-CF_3	169	167–169[e]	58	EtOH	C, H, N
3	2-Cl	192–193	*g*, *h*	45	MeOH	C, H, N
4	3-NO_2	210–211	209–210[f]	65	MeOH	C, H, N
5	2-CH=CH_2	177–178	172–173[e,h]	22	MeOH	C, H, N
6	2-NO_2*					
7	2-CH_3	181–183	*g*, *h*	22	MeOH	C,[c] H, N
8	2-Et*					C, H, N
9	2-Br	163–165		66	MeOH–H_2O	C, H, N
10	2-CN*		*g*, *h*			
11	3-Cl	190–192	*g*, *h*	55	MeOH	C, H, N
12	3-F	194	*h*	68	MeOH	C, H, N
13	H	198–199	197–198[g]	35	MeOH	C, H, N
14	3-CN	210	*h*	24	MeOH	C, H, N
15	3-I	184–185		56	MeOH–H_2O	C, H, N
16	2-F	207–208		26	MeOH–H_2O	C, H, N
17	2-I	179–180		48	MeOH	C, H, N
18	2-OMe	198–200	*g*, *h*	45	MeOH	C, H, N
19	3-CF_3	175–176	*g*	66	MeOH	C, H, N
20	3-CH_3	190–191	*h*	44	MeOH	C, H, N
21	2-OEt	140–142		28	EtOH–hexane	C, H, N
22	3-OCH_3	175–176	*g*, *h*	65	MeOH	C, H, N
23	3-NMe_2	182–183		44	MeOH	C, H, N
24	3-OH	227–229		22	MeOH	C, H, N
25	3-NH_2	216–217	214–216[f]	85	MeOH	C, H, N
26	3-OAc	168–169		95	MeOH–H_2O	C, H, N
27	3-OC(O)Ph	192–193		45	MeOH	C, H, N
28	2-NH_2	174–175	*f*	85	MeOH	C, H,[d] N
29	3-$N^+Me_3I^-$	204–205	*h*	95	MeOH	C, H, N
30	4-F	171	*h*	48	MeOH	C, H, N
31	4-Br	192		66	MeOH	C, H, N
32	4-I	220–221		74	MeOH	C, H, N
33	4-NO_2	198–199	196–197[f]	66	MeOH	C, H, N
34	4-NMe_2	189–191	*f*	25	MeOH	C, H, N
35	4-CN	232		79	EtOH	C, H, N
36	4-Cl	194–196	*g*, *h*	55	MeOH	C, H, N
37	2,6-Cl_2	238–240	*f*	5	MeOH	C, H, N
38	F_5	193–194	*h*	48	MeOH	C, H, N
39	2-F, 6-Cl	186–187	*h*	36	MeOH	C, H, N
40	2,3-Cl_2	185–187		44	MeOH	C, H, N
41	2-Cl, 5-NO_2	107		38	MeOH–hexane	C, H, N
42	3,5-Cl_2	192–193		28	MeOH	C, H, N
43	2-OH, 5-NO_2	217–219		11	MeOH	C, H, N
44	2,5-Me_2	164		15	EtOH–hexane	C, H, N
45	2,4-Cl_2	188–189		54	MeOH	C, H, N
46	2,4,5-(OMe)	172–173		45	MeOH	C, H, N
47	3-N_3*		*h*			
48	4-CH_3	174–175	*h*	66	MeOH	C, H, N

[a] All analyses agree within ±0.4% except as noted. [b] C: calcd, 53.69; found, 52.23. [c] C: calcd, 68.56; found, 68.10. [d] H: calcd, 6.95; found, 7.40. [e] Reference 7. [f] Bossert, F.; Vater, W. U.S. Patent 3 485 847, 1969. [g] Reference 9. [h] Reference 8. [i] (*) These compounds were donated by Bayer, A.G.

$B_{1_{para}}$ lowers the multiple correlation coefficient to 0.49. Adding the ortho steric term $B_{1_{ortho}}$ raised the correlation coefficient only to 0.90 but was not significant in terms of the *t* test. Substitution of σ_i, σ_i^2, σ_{omp}, or *F* for σ_m or L_{para} for $B_{1_{para}}$ also gave reasonably good correlations, but none was as good as that of eq 10.

Addition of the 10 polysubstituted compounds to the above data set together with one additional steric term produces eq 11.

$$\log 1/IC_{50} = 0.62\ (\pm 0.09)\pi + 1.96\ (\pm 0.29)\sigma_m - 0.44(\pm 0.09)L_{meta} - 3.26\ (\pm 0.33)B_{1_{para}} - 1.51\ (\pm 0.26)L_{meta'} + 14.23\ (\pm 0.78) \quad (11)$$

$$n = 46,\ r = 0.90,\ s = 0.67,\ F = 33.93$$

Again, inclusion of steric terms (B_1 or *L*) for ortho or ortho′ substituents raised the correlation coefficient insignificantly ($r = 0.91$) and gave regression coefficients for these terms which failed to satisfy the *t* test.

Equation 11 confirms that steric interactions are more unfavorable at the meta′ position than at the meta position. X-ray crystal structures of ortho- and meta-monosubstituted compounds show that the preferred conformer has the substituent oriented away from the dihydropyridine ring (exo), with the exception of the 3-cyano derivative in the nifedipine series.[3,4,18]

In all of our equations the value of σ_m is entered for all substituents without regard to their position of attachment to the aromatic ring. For the 46 compounds in this data set, there is a high correlation between σ_m and *F* or σ_I, $r = 0.96$ and $r = 0.95$, respectively. Although a more clear physical interpretation of the electronic substituent effect as an inductive one is obtained with *F* or σ_I, the use of σ_m was preferred since a greater number of such constants are available and improved correlations resulted from their use. Certainly there is no evidence for significant transfer of electronic charge between the dihydropyridine ring and the aromatic ring, and therefore there is no reason to reference σ values to the point of attachment of the two

(18) Fossheim, R.; Joslyn, A.; Solo, A. J.; Luchowski, E.; Rutledge, A.; Triggle, D. J. *J. Med. Chem.* **1988**, *31*, 300.

rings. We also note that other electronic descriptors including σ, F, and R give reasonable correlations both for our full set of compounds and for the various subsets.

These correlations consistently indicate that pharmacological data for nifedipine analogues is dependent on lipophilicity, an electronic term, and separate steric terms for each position on the aromatic ring. While the optimal description for the electronic and steric terms is arguable, the need for inclusion of each of these factors seems well established. The similarity in regression coefficients between eq 9, 10, and 11 tends to support the validity of these equations.

The dichotomy seen in comparing eq 10, which correlates the pharmacological data for all monosubstituted compounds, with Richards' equations 3 and 4, which correlate binding data, invited closer examination. Regression analysis of binding data for all monosubstituted compounds gave eq 12. This equation is based on a larger

$$\log 1/IC_{50} = 0.81\ (\pm 0.11)\pi + 2.36\ (\pm 0.51)\sigma_m + 0.99(\pm 0.35)B_{1_{ortho}} - 3.18\ (\pm 0.49)B_{1_{para}} + 9.83\ (\pm 0.80) \quad (12)$$

$$n = 21,\ r = 0.95,\ s = 0.49,\ F = 38.91$$

number of compounds than was used by Richards and gives an improved correlation coefficient. Because eq 3 and 4 contain more variables than is justified by the number of compounds being studied, they are of dubious statistical validity. A comparison of eq 12 to eq 10 reveals that the binding data shows a weakly positive dependence on the size of ortho substituents while the pharmacological data shows little correlation with that parameter. This is in accord with our finding that the pharmacological data for our ortho monosubstituted compounds gives a much weaker correlation with B_1 than that found for the binding data by Richards.

Correlation of the pharmacological data for the compounds in Richards' data set with his choice of variables gives an equation with $r = 0.956$. Inclusion of the 2-substituted bromo and iodo compounds in such a regression reduces the correlation coefficient to 0.926. Similar regression of the full set of 36 monosubstituted compounds further reduces the correlation coefficient to 0.649. This shows that the difference between the QSARs for the pharmacological data and the binding data does not arise primarily from inclusion of the bromo and iodo compounds but rather is a consequence either of the overly limited data base from which Richards derived his equations, of the data being derived from different tissues, or of differences in the structure–activity requirements for binding vs pharmacology. Currently, we are extending our study in an attempt to test our equations and to delineate the differences between requirements for binding and for pharmacological response.

Experimental Section

Chemistry. The 1,4-dihydropyridines listed in Table III were prepared by a standard Hantzsch procedure by refluxing methyl acetoacetate, ammonia, and the appropriate benzaldehyde in equimolar amounts in methanol for 2–24 h. Compounds were purified by recrystallization, and purities were established by melting points, thin-layer chromatography, NMR spectral characterization, and elemental analysis. The synthetic procedures are described in the literature.[19a]

The starting benzaldehydes were commercially available except for 2-vinylbenzaldehyde, which was prepared according to the procedure of Dale, Starr, and Strobel.[19b]

Pharmacology. Longitudinal muscle strips of guinea pig ileum were prepared to record mechanical responses. In brief, tissue pieces, approximately 2 cm long, were suspended in 10-mL jacketed glass tissue baths connected to a recirculating heated reservoir maintained at 37 °C. Each tissue was fixed to the bottom of the tissue bath with a stainless steel hook and the other end attached to the writing lever of a smoked drum kymograph. The physiologic saline (PSS) had the following composition (millimolar): NaCl, 118; KCl, 4.7; $MgCl_2$, 1.2; $CaCl_2$, 1.8; $NaHCO_3$, 84; KH_2PO_4, 1.2; dextrose, 5.5 and was aerated with O_2/CO_2 (95:5).

Isotonic recordings were made at a magnification ratio of 9:1 against 500 mg of resting tension. The contraction height of the phasic (fast) and tonic (slow) components of response was measured from the base-line tension attained by each tissue and recorded as millimeters contraction. Longitudinal smooth muscle strips were allowed to equilibrate for 90–120 min. During this time, the bathing solutions were replaced with fresh PSS every 15 min and the tissues were twice exposed at 60-min intervals to the muscarinic agonist methylfurmethide (MF) at 5.0×10^{-7} M. The magnitudes of the second responses were taken as control values. The tissues were equilibrated for a further 60 min, and the antagonist was then equilibrated for 30 min and a response to the MF was measured. Only one drug concentration was used on any given tissue. The pharmacological data represent the mean of 4–10 individual determinations. Standard errors are not greater than 5% of these mean values.

Statistics. Statistical analysis of pharmacologic data employed standard pharmacology programs implemented on an IBM PC. Multiple linear regression QSAR computations were performed with QSAR-PC.[20]

Acknowledgment. We are grateful to Michael Clark and Stephen P. Adams for their aid in the synthesis of nifedipine analogues. This work was supported, in part, by the National Institutes of Health (HL-16003).

Registry No. 1, 93515-16-3; 2, 53219-40-2; 3, 43067-01-2; 4, 21881-77-6; 5, 96689-65-5; 6, 21829-25-4; 7, 73257-44-0; 8, 116324-70-0; 9, 94817-50-2; 10, 73257-42-8; 11, 58395-01-0; 12, 86408-09-5; 13, 70677-78-0; 14, 32947-20-9; 15, 116324-71-1; 16, 86408-08-4; 17, 116324-72-2; 18, 73257-43-9; 19, 73257-46-2; 20, 80307-08-0; 21, 116324-73-3; 22, 43114-34-7; 23, 116324-74-4; 24, 116324-75-5; 25, 21835-63-2; 26, 116324-76-6; 27, 116324-77-7; 28, 21889-33-8; 29, 66941-33-1; 30, 86408-10-8; 31, 94889-62-0; 32, 116324-78-8; 33, 21829-09-4; 34, 21835-70-1; 35, 32927-12-1; 36, 73257-49-5; 37, 116324-79-9; 38, 75239-47-3; 39, 86408-11-9; 40, 91189-59-2; 41, 21829-30-1; 42, 116324-80-2; 43, 116324-81-3; 44, 116324-82-4; 45, 77233-94-4; 46, 116324-83-5; 47, 34686-43-6; 48, 73257-48-4.

(19) (a) Iwanami, M.; Shibanuma, T.; Fujimoto, M.; Kawai, R.; Tamayawa, K.; Takenaka, T.; Takahasi, K.; Murakami, M. *Chem. Pharm. Bull.* **1979**, *27*, 1426. (b) Dale, W. J.; Starr, L.; Strobel, D. W. *J. Org. Chem.* **1961**, *26*, 2225.

(20) Coburn, R. A. *QSAR-PC*; BIOSOFT, Cambridge, in press.