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QSAR of binding of dihydropyridine-type calcium antagonists to their receptor on ileal smooth muscle preparations

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Quantitative structure-activity analysis (Hansch analysis) is applied to elucidate the structural requirement for the binding of dihydropyridine-type calcium antagonists (DHPs) to their receptor in the guinea-pig ileal muscle preparations. It is found that various steric (B1, L), electronic (σ) and hydrophobic (π) parameters or their combinations correlate well with the potency of various DHPs to inhibit the binding of [^{3}H]nitrendipine to the microsomal preparations of the guinea-pig ileal muscle. The potency of DHPs increases with the minimum width (B1) of substituent at *ortho*- or *meta*-positions, but decreases with the increase in the length of substituent at the *meta*-position. The potency of DHPs decreases with the increase in both minimum width or length of substituent at the *para*-position and the optimal values were found to be those for hydrogen. The hydrophobicity (π) of substituents at different positions in the 4-phenyl ring affects the potency differently, indicating that a different environment exists around each position at the binding site. From the slopes of the π variable in the regression equations, it is concluded that the receptive environment of the *ortho*-position of the 4-phenyl ring of DHPs is lipophilic, and for that of the *para*-position hydrophilic. A good correlation is also observed between the Hammett electronic parameter (σ) and biological activity of *meta*-substituted DHPs. It is suggested that in the binding of the substituted 4-phenyl DHPs to their receptor, both electronic and hydrophobic interactions should be considered.

Of the calcium antagonists used as cardiovascular drugs, dihydropyridines (DHPs) or nifedipine analogues are among the most studied (Flecknestein 1983, Godfraind & Miller 1983, Spedding 1985). It is believed that they have a distinct binding site on the calcium channel of the membranes of various tissues (Glossman et al 1984); the binding of DHPs correlates well with their pharmacological activity (Triggle & Janis 1984).

The present study sought to apply Hansch analysis to elucidate structural requirements for the binding of DHPs to their receptor and thus to provide guidelines for the design of new drugs.

METHODS

Quantitative structural activity relationships (QSAR) were examined by computerized multiple regression analysis (Hansch 1973). The numerical values for the substituent parameters of substituted 4-phenyl DHPs were correlated with their potency in inhibiting the binding of [^{3}H]nitrendipine to the microsomal fraction of guinea-pig longitudinal ileal muscle (expressed as log 1/IC50; Bolger et al 1983).

For m DHP derivatives and n substituent parameters used, the entire set of equations can be written as follows:

$$y = \log (1/IC50) = K_0 + K_1 X_i + K_2 X_i + \ldots K_n X_i \quad (1)$$

the constants K_0 to K_n for n parameters in the regression equations were determined by multiple linear regression analysis. The validity of the regressions was judged by (i) standard deviation, s; (ii) F-test value; (iii) level of significance, *P*; (iv) correlation coefficient, r (see Hansch 1973; Tute 1971).

The physicochemical parameters were taken from Verloop et al (1976) and Norrington et al (1975), and biological data from Bolger et al (1983). Values used are tabulated in Table 1.

RESULTS

Hansch analysis detects quantitative relationships between the biological activity of derivatives and their physicochemical properties expressed by the substituent constants; it has been useful in understanding drug action on a molecular level and in designing new drugs (Tute 1971; Hansch 1973). The method has been applied here to the binding of

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Table 1. Data of substituent constants for *o-m-p*-substituted 4-phenyl 1,4-dihydropyridine analogues of nifedipine and of the biological activity, expressed as −log (IC50) values for inhibiting the binding of [^{3}H]nitrendipine to guinea-pig ileal preparations; [a] values from Bolger et al (1983), [b] values from Norrington et al (1975), [c] values from Verloop et al (1976).

	Analogue	−log(IC50)[a]	π[b]	σ[b]	B1[c]	L[c]
2,3,4	H	7·85	0·0	0·0	1·0	2·06
	2-CN	9·18	−0·33	—	1·6	4·23
	2-NO_2	9·08	−0·23	1·24	1·7	3·44
	2-Me	8·71	0·86	−0·13	1·52	3·00
	2-Cl	9·78	0·76	0·68	1·80	3·52
	2-MeO	7·87	−0·33	0·0	1·35	3·98
	2-F	8·44	0·0	0·54	1·35	2·06
	3-NO_2	9·97	0·11	0·71	1·7	3·44
	3-N_3	8·67	0·46	0·27	1·5	4·62
	3-MeO	7·27	0·12	0·12	1·35	3·98
	3-CN	8·68	−0·31	0·56	1·6	4·23
	3-Cl	9·30	0·77	0·37	1·8	3·52
	3-F	8·49	0·22	0·34	1·35	2·06
	3-Me	7·28	0·52	−0·07	1·52	3·00
	4-Cl	6·22	0·73	0·23	1·80	3·52
	4-Me	7·18	0·63	−0·17	1·52	3·00
	4-F	7·46	0·15	0·06	1·35	2·06
	4-NO_2	6·52	0·22	1·24	1·70	3·44

substituted 4-phenyl DHPs (Fig. 1, Table 1) to smooth muscle (Bolger et al 1983) either for each group of *ortho-*, *meta-* or *para*-substituted DHPs separately or as one set of data.

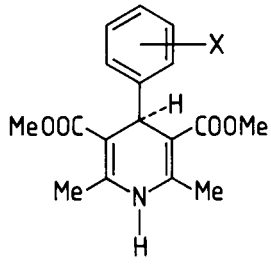

FIG. 1. Structure of nifedipine analogues used in this analysis.

Ortho-*substituted 4-phenyl DHPs*

The analysis for *ortho*-substituted DHPs shows that there is a significant correlation between biological activity and the minimum width of substituent (Verloop's B1 parameter) at the *ortho* position (eqn 2, Fig. 2).

$$y_o = 5{\cdot}152 + 2{\cdot}407\ B1_o \qquad (2)$$

$n = 7$, $r = 0{\cdot}91$, $s = 0{\cdot}32$, $F_{1,5} = 23{\cdot}81$, $P < 0{\cdot}05$

No significant correlation was observed for the parameters σ, π, or L (length of substituent) as single independent variables. The small number of analogues does not justify the use of more than one variable.

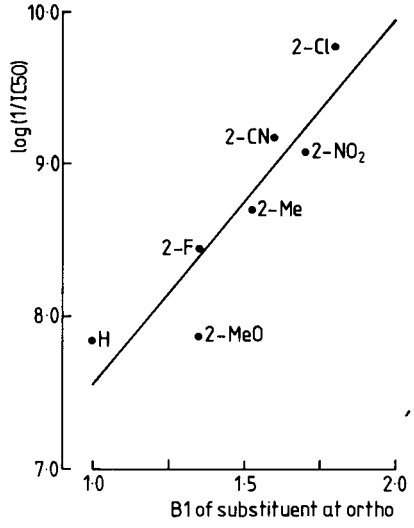

FIG. 2. A plot of the potency of various *ortho*-substituted 4-phenyl DHPs vs the minimum width (B1) of the *ortho*-substituent. The line is drawn using equation 2.

Meta-*substituted 4-phenyl DHPs*

Analysis reveals a good correlation between biological activity of substituted DHPs and Hammett constant, σ parameter, of the substituent at the *meta*-position (eqn 3, Fig. 3).

$$y_m = 7{\cdot}543 + 3{\cdot}116\ \sigma_m \qquad (3)$$

$n = 8$, $r = 0{\cdot}882$, $s = 0{\cdot}48$, $F_{1,6} = 21{\cdot}01$, $P < 0{\cdot}005$

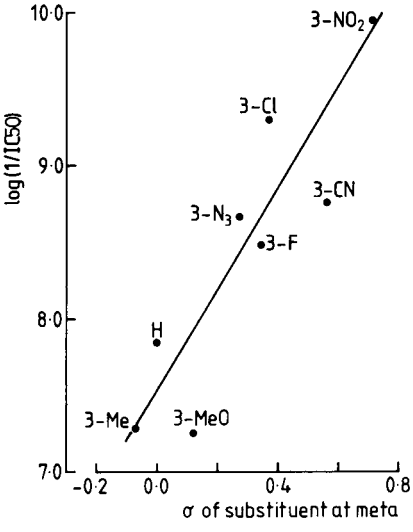

FIG. 3. A plot of the potency of various *meta*-substituted 4-phenyl DHPs vs the Hammet constant (σ) of the *meta*-substituent. The line is drawn using equation 3.

While no significant correlation could be observed for the steric parameters (B1, L) or hydrophobic constant (π), their combination with σ improved the correlation coefficient, r, over that of σ alone (eqns 4–6).

$$y_m = 7{\cdot}230 + 3{\cdot}444\,\sigma_m + 0{\cdot}927\,\pi_m \quad (4)$$

$n = 8$, $r = 0{\cdot}938$, $s = 0{\cdot}39$, $F_{2,5} = 18{\cdot}18$, $P < 0{\cdot}001$

$$y_m = 6{\cdot}931 + 2{\cdot}846\,\sigma_m + 0{\cdot}467\,B1_m \quad (5)$$

$n = 8$, $r = 0{\cdot}887$, $s = 0{\cdot}516$, $F_{2,5} = 9{\cdot}24$, $P < 0{\cdot}025$

$$y_m = 7{\cdot}919 + 3{\cdot}263\,\sigma_m - 0{\cdot}124\,L_m \quad (6)$$

$n = 8$, $r = 0{\cdot}890$, $s = 0{\cdot}51$, $F_{2,5} = 9{\cdot}5$, $P > 0{\cdot}025$

Different steric parameters of substituents at the *meta*-position affect the biological activity in a different manner. While the increase in minimum width of substituent (B1) increases the activity, the increase in the length of substituent (L) decreases the activity.

Para-*substituted 4-phenyl DHPs*

The analysis shows that there is a good correlation (but with negative slope) between the potency of *para*-substituted DHPs to inhibit [^{3}H]nitrendipine binding, and either the minimum width (B1) or the length (L) of the substituent at the *para*-position (eqns 7, 8, Fig. 4).

$$y_p = 10{\cdot}051 - 2{\cdot}039\,B1_p \quad (7)$$

$n = 5$, $r = 0{\cdot}96$, $s = 0{\cdot}21$, $F_{1,3} = 37{\cdot}02$, $P < 0{\cdot}01$

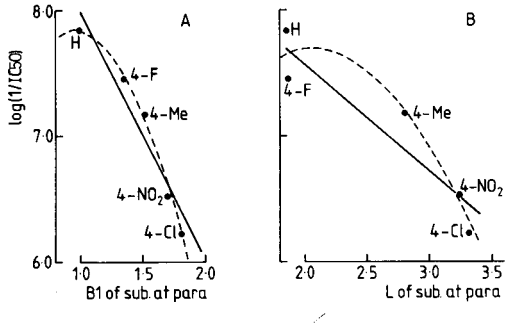

FIG. 4. Plots of potency of various *para*-substituted 4-phenyl DHPs vs either the minimum width (part A) or the length (part B) of the *para*-substituent. The solid line is drawn using equations 7 (part A) or 8 (part B). The broken line is drawn using equations 9 (part A) and 10 (part B).

$$y_p = 9{\cdot}512 - 0{\cdot}876\,L_p \quad (8)$$

$n = 5$, $r = 0{\cdot}94$, $s = 0{\cdot}27$, $F_{1,3} = 22{\cdot}27$, $P < 0{\cdot}025$

No significant correlation was observed between either σ or π parameters of the *para*-substituent and biological activity.

While the small number of analogues available for the analysis does not justify a firm statement, the addition of $B1^2$ or L^2 to the above equations greatly improves the value of r and allows calculation of optimum values for the B1 and L of the substituent at *para*-position (eqns 9, 10. Fig. 4).

$$y_p = 5{\cdot}683 + 4{\cdot}506\,B1_p - 2{\cdot}344\,B1^2_p \quad (9)$$

$n = 5$, $r = 0{\cdot}998$, $s = 0{\cdot}028$, $F_{2,2} = 222{\cdot}02$, $P < 0{\cdot}005$

$$y_p = 3{\cdot}238 + 3{\cdot}951\,L_p - 0{\cdot}877\,L^2_p \quad (10)$$

$n = 5$, $r = 0{\cdot}976$, $s = 0{\cdot}206$, $F_{2,2} = 20{\cdot}19$, $P < 0{\cdot}05$

The optimum values of B1 and L are 0·96 and 2·25, respectively, values similar to those for H (B1 = 1·0 and L = 2·06).

Substituted 4-phenyl DHPs as one set of data

Analysis of data for the whole set of compounds was more complicated, as can be seen from the foregoing results which show that the same substituent at different positions affects the biological activity in a different manner. This was confirmed by the fact that no significant correlation was observed for any single steric, hydrophobic (π) or electronic (σ) parameter included in the analysis without considering the position of substituent. However, when the value of the hydrophobic constant (π) for each position is treated as a separate parameter, a good correlation is observed between the hydrophobic constant of substituent at *ortho*-(π_o) and *para*-(π_p) position of the phenyl ring and the biological activity (eqn 11).

$$y = 8{\cdot}491 + 0{\cdot}636\,\pi_o - 3{\cdot}146\,\pi_p \quad (11)$$

$n = 18$, $r = 0{\cdot}67$, $s = 0{\cdot}85$, $F_{2,15} = 6{\cdot}13$, $P < 0{\cdot}025$

Whereas introduction of hydrophobic constants for the substituent at *meta*-positions of the phenyl ring did *not* improve the correlation, the addition of σ of the substituent at *meta* did improve the correlation (eqn 12).

$$y = 8{\cdot}209 + 0{\cdot}766\,\pi_o - 2{\cdot}647\,\pi_p + 1{\cdot}799\,\sigma_m \quad (12)$$

$n = 18$, $r = 0{\cdot}763$, $s = 0{\cdot}77$, $F_{3,14} = 6{\cdot}48$, $P < 0{\cdot}01$

A good correlation was also observed for the steric parameters (eqns 13, 14).

$$y = 6{\cdot}349 + 2{\cdot}638\,B1_{o,m} - 0{\cdot}197\,L_m - 1{\cdot}144\,B1_p \quad (13)$$

$n = 18$, $r = 0{\cdot}882$, $s = 0{\cdot}56$, $F_{3,14} = 16{\cdot}33$, $P < 0{\cdot}001$

$$y = 6{\cdot}322 + 2{\cdot}735\,B1_{o,m} - 0{\cdot}194\,L_m - 0{\cdot}622\,L_p \quad (14)$$

$n = 18$, $r = 0{\cdot}888$, $s = 0{\cdot}55$, $F_{3,14} = 17{\cdot}34$, $P < 0{\cdot}001$

Separation of minimum width of the substituent at position *ortho*- or *meta*- into two separate terms did

not improve the correlation nor change the slopes of the variables to a significant extent.

Addition of the electronic constant of the substituent at the *meta*-position (σ_m) greatly improved the correlation (eqns 15, 16).

$$y = 7{\cdot}566 + 2{\cdot}238\ B1_{o,m} - 0{\cdot}479\ L_m - 1{\cdot}288\ B1_p + 1{\cdot}948\ \sigma_m \quad (15)$$

$$n = 18,\ r = 0{\cdot}93,\ s = 0{\cdot}45,\ F_{4,13} = 21{\cdot}27,\ P < 0{\cdot}001$$

$$y = 7{\cdot}430 + 2{\cdot}376\ B1_{o,m} - 0{\cdot}472\ L_m - 0{\cdot}674\ L_p + 1{\cdot}928\ \sigma_m \quad (16)$$

$$n = 18,\ r = 0{\cdot}93,\ s = 0{\cdot}43,\ F_{4,13} = 23{\cdot}10,\ P < 0{\cdot}001$$

DISCUSSION

The present study provides quantitative structure-activity relationships for the potency of different 4-phenyl DHPs (nifedipine analogues) in inhibiting the [^{3}H]nitrendipine binding to DHPs receptors on the membrane of guinea-pig ileal smooth muscle (Bolger et al 1983).

Among various QSAR procedures, the Hansch method has been the most widely and effectively used (Tute 1971; Hansch 1973). It assumes that the potency of biological activity exerted by a series of congeneric compounds is expressible in the terms of a function of various physicochemical parameters of the compounds. If a function could be formulated showing that certain physicochemical properties are favourable or unfavourable to the activity, then the structural modifications could be planned to generate compounds of more desirable activity.

Although DHPs are among the best known groups of calcium antagonists, only a limited number of studies on their QSAR have been carried out (Rodenkirchen et al 1979; Triggle et al 1980; Fossheim et al 1982; Seidel et al 1984).

Rodenkirchen et al (1979) have shown that a good and positive correlation exists between the negative inotropic affect of different DHPs on the cardiac muscle and the minimum width of the substituent at the *ortho*-position of the 4-phenyl ring. A good but negative correlation was also observed with hydrophobic or steric parameters of substituents of 3,5-ester residues.

The effect of steric parameters also has been shown by Triggle et al (1980) who found a good correlation between the extent of 1,4-DHP ring pucker (calculated from solid-state structures) and the pharmacological activity of some DHPs.

Similarly, by measuring KI values of some rigid nifedipine analogues in a receptor binding assay ([^{3}H]nimodipine displacement), Seidel et al (1984) have shown that activity increases when the deviation of the phenyl ring from a position bisecting the plane of DHP ring is reduced. They concluded that interaction with the receptor involves a preferred conformation where the phenyl ring is in a perpendicular orientation to the DHP ring.

Our own analysis confirms the finding of Rodenkirchen et al (1979) in that minimum width of substituent at the *ortho*-position (equations: 1, 13–16, Fig. 2) or *meta*-position (eqns 5, 13–16) of the 4-phenyl ring, correlate well with the activity of DHPs. Loev et al (1974) have suggested that the biological activity of the aryl-substituted derivatives may be enhanced by the presence of bulky groups which causes the 4-phenyl ring to prefer an orientation perpendicular to the plane of the dihydropyridine ring. Rodenkirchen et al (1979) further proposed that an optional drug-receptor interaction is only possible when the *ortho*-aryl substituent obtains the highest degree of spatial complementarity to the active site of the receptor.

Our analysis shows that this spatial complementarity is not restricted to the *ortho*-position only, but also involves *meta*- and para-positions. The requirements are:

(1) a bulky substituent at the *ortho*-position; activity increases with the increase in the minimum width of substituent at *ortho*-position;

(2) a wide but not lengthy substituent at the *meta*-position; activity increases with the increase in minimum width of substituent, but decreases with the increase in the length of substituent at the *meta*-position;

(3) a small (preferably H) substituent at the *para*-position; activity decreases with the increase in both the minimum width and length of substituent at the *para*-position.

Our analysis also revealed something about the environment of the binding site, since a positive correlation was observed for the hydrophobicity of substituent at the *ortho*-position and a negative one for the *para*-position. This indicates that the environment around the *ortho*-position is lipophilic and that round the *para*-position is hydrophilic. The border of these two phases would be expected at the *meta*-position and that is confirmed by the fact that hydrophobicity of the *meta*-substituent does not have a great influence on the binding of DHPs. These results are summarized in Fig. 5.

While Rodenkirchen et al (1979) have reported that electronic parameters of substituents do not correlate with the negative inotropic effect of DHPs on cardiac muscle in-vitro, we have found that a good

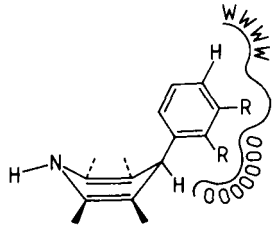

FIG. 5. The receptor environment around the 4-phenyl ring of DHP calcium antagonists in the guinea-pig ileal smooth muscle; 'w' and 'o' represent the hydrophilic and lipophilic environments, respectively.

correlation exists between σ of the substituent at the *meta*-position and the potency of different DHPs to inhibit the binding of [^{3}H]nitrendipine to ileal smooth muscle preparations. While the number of analogues reported by Rodenkirchen et al (1979) is small, there is some correlation between the σ parameter of the substituent at the *meta*-position and negative inotropic effects of DHPs on cardiac muscle (eqn 17).

$$y = 5{\cdot}790 + 0{\cdot}523\ \sigma_m \qquad (17)$$

$n = 5$, $r = 0{\cdot}82$, $s = 0{\cdot}11$, $F_{1,3} = 6{\cdot}42$, $P < 0{\cdot}1$

Good agreement is observed for the slopes of various parameters between those which are calculated from a selected group of analogues (e.g. using only *ortho*-, *meta*- or *para*-substituted analogues) and those which are calculated from the whole data set.

The slope for the B1 value of substituents at the *ortho*-position of the 4-phenyl ring of DHPs, calculated from the binding data of Bolger et al (1983), was higher than that reported by Rodenkirchen et al (1979) calculated from the effect of DHPs on cardiac muscle. Triggle & Janis (1984) have shown that while the pharmacological effect, or the binding, of various DHPs in cardiac muscle correlates with their binding to the ileal smooth muscle preparation, the slope of this correlation deviates from the value of one. This indicates that the DHP receptors in these two tissues are not quite the same. Therefore, the above variation in the slope of B1 of substituents at the *ortho*-position of the phenyl ring may result from the difference in the structural requirements for the binding of DHPs to different tissues. Further analysis with more analogues is required to clarify this point.

In conclusion, our QSAR analysis shows that, for the binding of various DHPs to their receptor on the ileal smooth muscle, both electronic and hydrophobic interactions are involved. The structural requirements for optimal binding are: (i) a hydrogen at the *para*-position, (ii) a bulky and lipophilic group at the *ortho*-, and (iii) a bulky group with high Hammett constant, σ, at the *meta*-position of the 4-phenyl ring. Whereas the geometry of the DHP receptor of smooth muscle is similar to that of cardiac muscle, it is not identical to it.

REFERENCES

Bolger, G. T., Gengo, P., Klockowski, R., Luchowski, E., Siegel, H., Janis, R. A., Triggle, A. M. Triggle, D. J. (1983) J. Pharmacol. Exp. Ther. 225: 291–309

Flecknestein, A. (1983) Circ. Res. 52 (suppl. 1): 3–16

Fossheim, R., Svarteng, K., Mostad, A., Romming, C., Shefter, E., Triggle, D. J. (1982) J. Med. Chem. 25: 126–131

Glossmann, H., Ferry, D. R., Goll, A., Rombusch, M. 1984 J. Cardiovasc. Pharmacol. 6 (suppl. 4): S608–S621

Godfraind, T., Miller, R. C. (1983) Circ. Res. 52 (suppl. 1): 81–91

Hansch, C. (1973) in: Structure-activity relationships. Cavallito, C. J. (ed.) Pergamon Press, Oxford, Vol. 1

Hansch, C., Leo, A., Unger, S. H., Kim, K. H., Nikaitani, D., Lien, E. J. (1973) J. Med. Chem. 16: 1207–1216

Loev, B., Goodman, M. M., Snader, K. M., Tedeschi, R., Macko, E. (1974) Ibid. 17: 956–965

Norrington, F. E., Driscoll, J. S., Hansch, C. (1975) Ibid. 18: 604–607

Rodenkirchen, R., Bayer, R., Steiner, R., Boggert, F., Meyer, H., Moller, E. (1979) Naunyn-Schmiedebergs Arch. Pharmacol. 310: 69–78

Seidel, W., Meyer, H., Born, L., Kazda, S., Dompert, W. (1984) Proc. 5th Eur. Symp. on QSAR, Bad Segeberg, pp 366–369

Spedding, M. (1985) Trends in Pharmacol. Sci. 6: 109–114

Triggle, D. J., Janis, R. A. (1984) in: Spector, S., Back, N. (eds) Modern Methods in Pharmacology. Alan R. Liss Inc., New York, Vol. 2, pp 1–28

Triggle, A. M., Shefter, E., Triggle, D. J. (1980) J. Med. Chem. 23: 1442–1445

Tute, M. (1971) Drug Res. 6: 1–77

Verloop, A., Hoogenstraaten, W., Tipker, J. (1976) in: Ariens, E. J. (ed.) Drug Design. Academic Press, New York, Vol. 7, pp 165–207